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14 Bioavailability and Ecotoxicity of PAHs

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Despite the apparent similarity in structure, PAHs should not be regarded as a homogeneous group of chemicals. The environmental chemistry and toxicology of PAHs is highly complex and does not depend on simple physico-chemical characteristics. The keys to an understanding of the environmental risk of PAHs are knowledge of the fraction available to the organism and the toxicity mechanisms involved, as these ultimately determine the magnitude and nature of biological effects.

The best way to assess PAH bioavailability is to determine the uptake rate constant via a kinetic study. This is, however, quite laborious and usually bioavailability is assessed on the basis of tissue residues. It is obvious that such an assessment can only be made if sufficient knowledge is available on kinetics and mechanisms of uptake, biotransformation and elimination, as large variations exist among different taxa of the animal kingdom in these mechanisms. Many biological factors also contribute to the within-species variability of toxicokinetics of PAHs.

Data from the scientific literature demonstrate that PAHs do not have one type of toxic action but that different toxicity mechanisms play a role, depending on the compound, the type of exposure (acute or chronic), the organism and the environmental conditions involved. The toxicity mechanisms that have been postulated for PAHs include the following.

Nonpolar narcosis, which is an aspecific mode of toxicity considered to be caused by the physical disturbance of the structure of the biological membrane. Narcosis can develop relatively quickly during short term exposure and is a phenomenon which is typically observed in acute toxicity experiments.

Phototoxicity, the term used for the phenomenon of increased toxicity of certain PAHs in the presence of UV light. The light-induced formation of free radicals and subsequent damage to a variety of macromolecules is believed to be responsible for this toxicity mechanism. This type of toxicity can develop relatively quickly and can be observed both in acute and chronic toxicity experiments.

Biochemical activation and subsequent adduct formation. During enzymatic transformation certain PAHs are transformed into highly reactive compounds which may form covalent bonds with macromolecules such as protein and DNA, the adducts. DNA adducts may give rise to mutations and this may result in carcinogenesis and teratogenesis, typical parameters of chronic toxicity experiments which take a long time to develop.

Disturbance of hormone regulation. There is only circumstantial evidence for this mechanism of toxicity, which is thought to occur either by direct interaction of PAH metabolites with hormone receptors (mimicking of hormones) or, indirectly, by interference with hormone metabolism. This type of toxicity will take some time to develop and will require chronic exposure.

Organisms which metabolize PAHs extensively, will be less susceptible to narcosis and phototoxicity of PAHs compared to organisms which do not metabolize PAHs, since the concentrations at the target site will be lower. At the same time, however, organisms that have a high rate of metabolism of PAHs are likely to be the victims of adduct formation or disturbance of the hormone regulation. Consequently, the relationship between residue levels of PAHs and toxicity is ambiguous. Clearly species specific information should be used when considering the relationship between residue level of PAHs and the possible risk to organisms.

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Data on the toxicity of PAHs and studies on the mechanisms behind this toxicity indicate that many PAHs can act through a more specific toxicity mechanism than nonpolar narcosis. Exceptions to this may be PAHs such as naphthalene, phenanthrene and fluorene, which are non-phototoxic, are not considered to be mutagens and do not have a structure similar to hormones. These compounds are quite abundant in the environment but are susceptible to biodegradation. PAHs with a higher molecular weight generally have longer residence times and have a more specific toxicological nature which may result in adverse effects during chronic exposure.

An overview of studies in which adverse effects of PAHs on reproduction, growth or survival are documented, reveals a considerable bias for short term toxicity studies. Under such conditions nonpolar narcosis is the most likely mechanism of toxicity to be observed. Effects due to other toxicity mechanisms, such as biochemical activation and disturbance of hormonal regulation, will be missed, as it will take a longer exposure period for effects to be observed at the individual level. Observations of phototoxicity are unlikely as the light sources used in most experiments are not representative of outdoor conditions. Under natural conditions, with long term exposure of organisms to PAHs, more specific, and therefore higher, toxicity can be expected than has generally been documented in the literature. The ecotoxicological consequences of PAH exposure can therefore not be properly assessed until more chronic data become available. In addition, a theoretical framework needs to be developed to deal with such long term chronic effects as carcinogenesis and mutagenesis, as the consequences of these on the population and ecosystem level are still poorly understood.

The potential risk for biota of a PAH contamination is usually assessed by comparing field concentrations with toxicity data from laboratory experiments. When the concentrations are so high that a significant potential risk for biota exists, one should focus on the actual risk. The latter takes into account reductions in bioavailability which may occur in the natural environment due, for example, to aging. The in-situ tissue residues of PAHs or their metabolites can be used as a measure of the bioavailability and mobility of contaminants, provided species specific information is taken into account.

Biological effects of a mixture of contaminants at a field site can be assessed using biochemical markers, which offer some diagnostic value. A disadvantage of biochemical markers is that these detect changes at the biochemical level, which may not be indicative of adverse health effects per se, as these effects may be within the tolerance limits of organisms. The development of a PAH specific suite of biomarkers is an important step forward in the assessment of the biological effects of PAH contaminated field sites. The success of such an approach hinges on field experience and on the development of an interpretational framework defining at what stage biochemical effects change from harmless to harmful.

Keywords: toxicity, PAHs, bioaccumulation, mode of action.

Contents

14.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are often regarded as a homogenous group of chemicals. As a consequence, the environmental risk of PAHs is simplified to that of a few well-known representatives, or ultimately to that of the summed concentrations of these representatives (ΣPAH). From experimental studies with PAHs evidence is however accumulating that the environmental chemistry and toxicology of PAHs is much more complex. It has been demonstrated that PAHs differ strongly in their physico-chemical properties such as water solubility, volatility, lipophilicity [Mackay and Callcott, Chap. 8] and susceptibility to photochemical [Arey, Chap. 9] and biochemical degradation [Neilson and Allard, Chap 10]. This results in significant differences among PAHs in their distribution in aquatic systems, the atmosphere and soil or sediment. This chapter on bioavailability and ecotoxicity of PAHs focuses on the partitioning of PAHs to biota, how biological characteristics affect this distribution and what are the toxicological consequences of PAH exposure for biota. This chapter attempts to give a comprehensive overview of the complex

interactions of PAHs with biota. It highlights the general patterns that have been recognized to date, and selects those procedures that may be valuable for assessment of the severity of PAH contamination in the field.

Environmental scientists have traditionally measured the PAH residues in organisms when they were confronted with PAH pollution of the environment. This has resulted in a vast literature on residue levels in biota, and the PAH residues often contain important information for the assessment of the environmental risk of PAH contamination. PAH concentrations within organisms are thought to reflect both the availability of PAHs in environmental matrices for uptake by biota, and the internal exposure concentration close to the site of toxic action. Both species specific and compound specific characteristics however determine the rate of elimination of PAHs from the tissue of biota and have a strong modifying effect on residue levels of PAHs in biota [212, 230]. Some important factors influencing residue levels in biota are reviewed in Sect. 14.2 and are crucial for a proper interpretation of tissue residues.

It is often stated that body residues reflect the internal concentration close to the site of toxic action, but what toxic action is meant here? There is increasing evidence for important differences among PAHs in the site of toxic action (toxicity mechanism), and four different toxicity mechanisms have been postulated for PAHs. Data from the scientific literature demonstrate that PAHs do not have a single type of toxic action but that different toxicity mechanisms play a role and depend on the compound, the type of exposure (acute or chronic), the organism and the environmental conditions involved. Section 14.3 focuses on the different toxicity mechanisms.

Another important instrument in the assessment of PAH contamination is the comparison of field concentrations with effect concentrations reported from laboratory toxicity studies. An overview of the ecotoxicological studies on PAHs is given in Sect. 14.4 and a bias in the ecotoxicological data set on PAHs is revealed. The data set was used to investigate (1) evidence for specific toxicity mechanisms, (2) the magnitude of sublethal relative to acute effect concentrations and (3) the sensitivity of marine vs fresh water species.

Based on the overview of bioavailability and toxicity that is presented, the final part of this chapter focuses on the assessment of contaminated field sites. Three steps can be recognized in the assessment process: (1) an assessment of the potential risk, based on a comparison of field concentrations with toxicity data obtained from laboratory toxicity experiments; (2) the assessment of the actual risk by estimating the bioavailability of the contaminant; and (3) the measurement of biological effects.

14.2 Factors Influencing Residue Levels

14.2.1 Introduction

Residue levels of PAHs in organisms are a result of a variety of processes, the most important of which are the partitioning between and within biotic and

abiotic compartments, and simultaneous transformation reactions such as (bio)degradation and (bio)transformation. As described in this and other chapters, order of magnitude differences may be present between individual PAHs in both physico-chemical characteristics (water solubility, volatility, hydrophobicity, photodegradation) and biological activity, e.g. microbial degradability, toxicokinetics, biotransformation, mutagenicity and carcinogenicity [103, 132, 150, 230]. Without a proper understanding of the dynamics of the underlying processes and mechanisms, interpretation of tissue residues in organisms may lead to erroneous conclusions.

In aquatic ecosystems, the fraction of freely dissolved PAHs, which is usually assumed to be available for uptake by most organisms, decreases rapidly with increasing hydrophobicity and with increasing concentrations of substrates rich in absorption sites (e.g. suspended matter, dissolved organic carbon). The actual bioavailability of PAHs in the water column and in interstitial water of sediments may be limited, especially for compounds with log $K_{ow} > 5$ [82]. From recent studies, evidence has emerged that prolonged contact times of PAHs with sediments and soils ('aging') may result in a markedly reduced bioavailability [23]. In addition, other factors, such as primary production, seasonal factors, hydrology, sedimentation, (bio)turbation, and other habitat-specific factors may influence the composition and partitioning of organic material [82].

In terrestrial habitats, PAHs are bound predominantly to the organic soil fraction. The stratification of soils in litter, fragmentation, humus and mineral layers results in large differences in the characteristics and the content of the organic fraction in soil profiles. The highest PAH concentrations can usually be found in the humus layers [319]. Together with the large fluctuations in water content of soils, due to rainfall, evaporation and drainage, it is likely that partitioning processes of PAHs with soil organic carbon are highly dynamic and that non-equilibrium conditions seem to predominate.

As has been indicated by various authors [129, 230, 343], large variations exist among different taxa of the animal kingdom in the mechanisms and kinetics of uptake, elimination and biotransformation of PAHs and other contaminants, sometimes even between closely related species. Many other biological factors (e.g. life stage, body weight, lipid weight, moulting, feeding habits, reproduction) may contribute to the intra-species variability of toxicokinetics of PAHs. The interactions of biotic and abiotic factors further limit the predictability of bioavailability and biotic concentrations in natural systems.

In the next sections, a brief introduction will be given to the factors that control bioaccumulation of PAHs in organisms. Emphasis is given to benthic invertebrates, as the highest PAHs concentrations are usually found in soils, sediments and organisms at lower trophic levels (algae, macrophytes and invertebrates). Vertebrates (mammals, birds, fish) generally have low tissue residues due to MFO-mediated biotransformation of PAHs, as discussed by De Maagd and Vethaak, Chap. 15. Although the a priori applicability of the equilibrium partitioning theory seems limited for complex and dynamic processes in the field situation, it will be used as an interpretational framework. Additionally, this chapter focuses mainly on unsubstituted parent PAHs, as these compounds have been studied most intensively. For the large and important number of

alkylated sulfur-, oxygen- or nitrogen-containing polycyclic aromatic compounds, and for PAHs with more than seven rings, the body of knowledge is still too limited for even preliminary risk-assessments.

14.2.2 Bioavailability

The processes that determine the fate of PAHs in aquatic ecosystems are indicated in Fig. 1. Abiotic partitioning and transformation reactions, such as biodegradation and photolysis, determine the actual ambient concentrations of PAHs to which organisms are exposed. The partitioning behaviour of PAHs between overlying or porewater, soils, sediments, particulate or colloidal matter and dissolved organic material is one of the major factors influencing the abiotic components of bioavailability, and has been documented in several studies [71, 151, 173, 202, 203, 213, 265]. As discussed in a previous chapter [Mackay and Callcott, Chap. 8], the binding affinity to these substrates is determined mainly by their hydrophobicity (e.g. K_{ow}, n-octanol/water partition coefficient) and by the content and characteristics of organic material of the binding substrates [151, 202, 203].

Uptake of PAHs by organisms may take place from aqueous systems via gills or the skin, and from dietary sources via the gastro-intestinal tract. Epibenthic and sediment inhabiting invertebrates may have additional uptake from ingested sediments or from porewater. Patterns of PAHs in sediment or particulate matter can be quite different from those in food items used by biota or in the free dissolved fraction [33, 54]. The fraction of a contaminant concentration that is available for uptake by aquatic organisms, i.e. the bioavailable fraction, varies between species and depends on the relative significance of different uptake pathways [23, 308, 329]. Therefore, bioavailability has always to be related to a (group of) species in distinct micro-environments with specifically defined route(s) of uptake.

Although various definitions of the concept of bioavailability have been proposed [6, 68], we prefer to use the more general phrasing, analogous to the definition used in pharmacokinetic studies, described by Belfroid et al. [23]: "the fraction of the chemical present in soil/sediment and (interstitial) water which can potentially be taken up during the organisms life-time into the organism tissue".

The best way to measure PAH availability is to determine the uptake rate parameters from kinetic studies. This is however quite laborious, and bioavailability is usually assessed from tissue residues. Tissue residue concentration of contaminants in organisms depends on the relative magnitude of the rates of uptake and elimination of the parent compound and/or biotransformation to metabolites. For neutral compounds, that are not or slowly biotransformed, the bioconcentration from aqueous sources is usually determined by the lipid content of the organism and the hydrophobicity of the compounds [46, 205]. The in-situ assessment of bioavailability is usually derived from tissue residues determined in sentinel species in passive [82, 175, 238, 280, 303] or active biomonitoring programs [25]. It is obvious, that such an assessment can only be

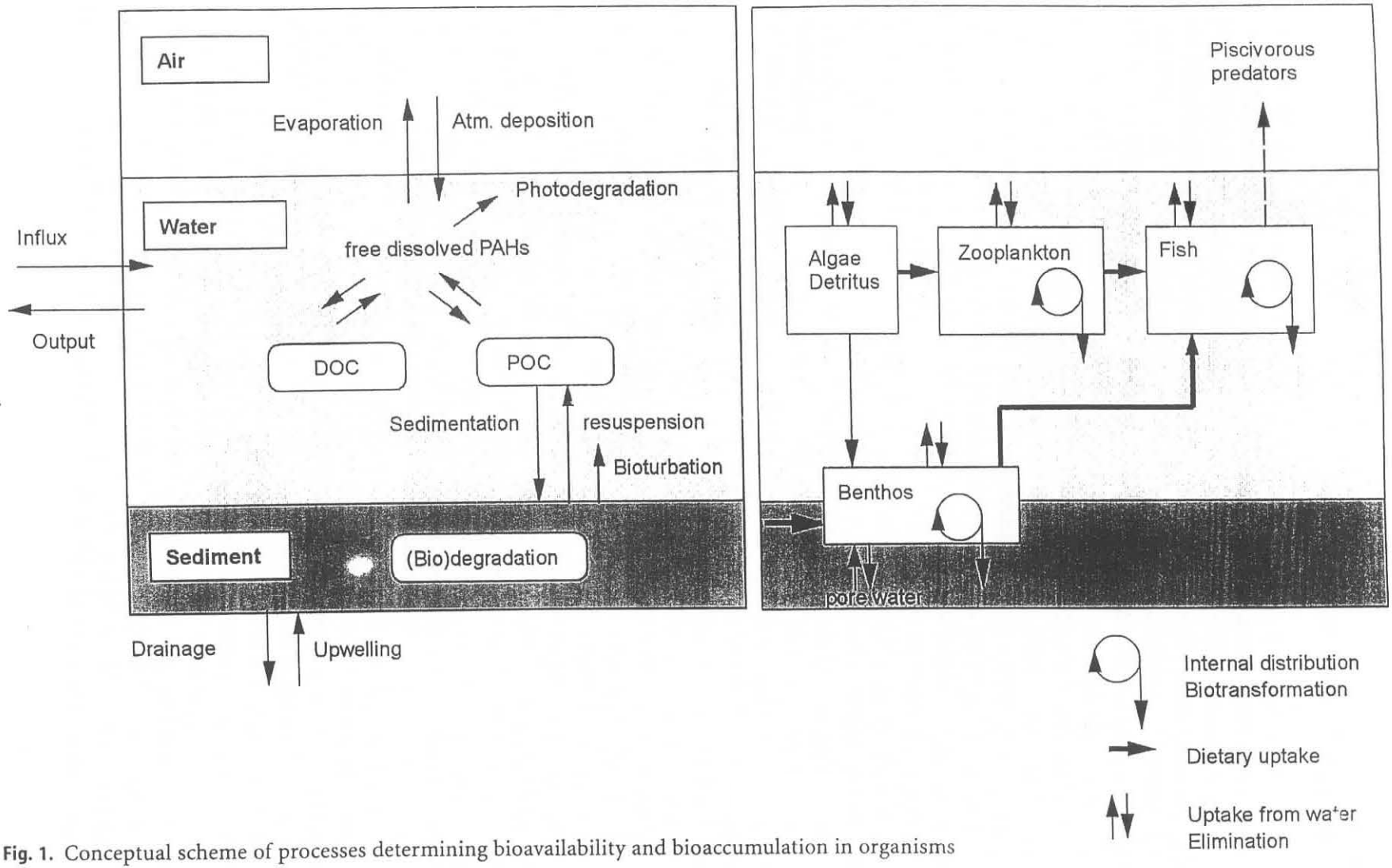

Fig. 1. Conceptual scheme of processes determining bioavailability and bioaccumulation in organisms

made if sufficient knowledge is available on the kinetics and the mechanisms of uptake, biotransformation and elimination.

14.2.2.1
Dissolved and Sediment Organic Carbon

Various well known case-studies on the influence of dissolved organic carbon (DOC), suspended matter and sediment organic carbon on the bioavailability of PAHs, halogenated PAHs including PCBs, PCDDs, PCDFs, and trace metals have been documented in the literature. At common levels of DOC in coastal and inland waters (1–10 mg/l), more than 60–95% of the hydrophobic five- and six-ring PAHs may be present as DOC-bound PAHs. For two- and three-ring PAHs this may range from 15–60%. For super hydrophobic compounds (log $K_{ow} > 7$) this fraction could exceed 95% in natural waters [82]. Readman et al. [265] presented data from a field study in the Tamar estuary (UK) in which similar binding patterns of PAHs to suspended particulate matter was demonstrated. As a result of the competing sorption processes and the variation in binding affinity among different PAHs, the less hydrophobic PAHs may be expected to be more available for uptake by organisms.

McCarthy et al. [203] demonstrated in a laboratory study that the bioconcentration factor (BCF) of 3-methylcholanthrene (an alkylated PAH) in *Daphnia magna* was decreased by almost 60% at concentrations of 1.5 mg/l dissolved humic materials (DHM) and that at levels of 15 mg/l dissolved humic materials a further reduction to almost 80% could be observed, compared to solutions free of dissolved humic materials. In various experimental studies similar reductions of bioaccumulation or toxicity of PAHs and polychlorinated PAHs in different species have been noted [167, 171, 173, 187, 200, 202, 212, 262].

The differences in sorption behaviour and bioavailability between individual PAHs usually result in typical variations in PAH profiles in different environmental compartments. An example of the shifting patterns of PAHs in various aquatic compartments is presented in Fig. 2, from a monitoring study in one of the sedimentation basins of the European rivers Rhine and Meuse [54]. The loading of PAHs of these rivers is relatively high, and still exceeds current quality objectives [137, 264]. The lower molecular weight PAHs seem to prevail in the dissolved water phase (including dissolved organic carbon), whereas PAHs with four and more rings predominate in the sediment. The pattern in suspended matter seems to have an intermediary position (Fig. 2). The relatively enhanced concentrations of high molecular weight PAHs in the water phase are caused by the co-sampling of PAHs bound to dissolved organic carbon (DOC), due to the operationally defined cut-off diameter (0.45 m) of the filter procedure used [329]. Determination of DOC-bound PAHs in natural waters can be done using C-18 preconcentration-columns or similar devices [168], but this method is not yet routinely applied in monitoring studies. In the patterns of the invertebrates (*Asellus aquaticus*) naphthalene, pyrene and fluoranthene are elevated. In related laboratory uptake-elimination studies the bioconcentration in freshwater isopods of PAHs with log $K_{ow} < 5$ appeared to be related to the hydrophobicity of the individual PAHs [329] and the biotransformation in this

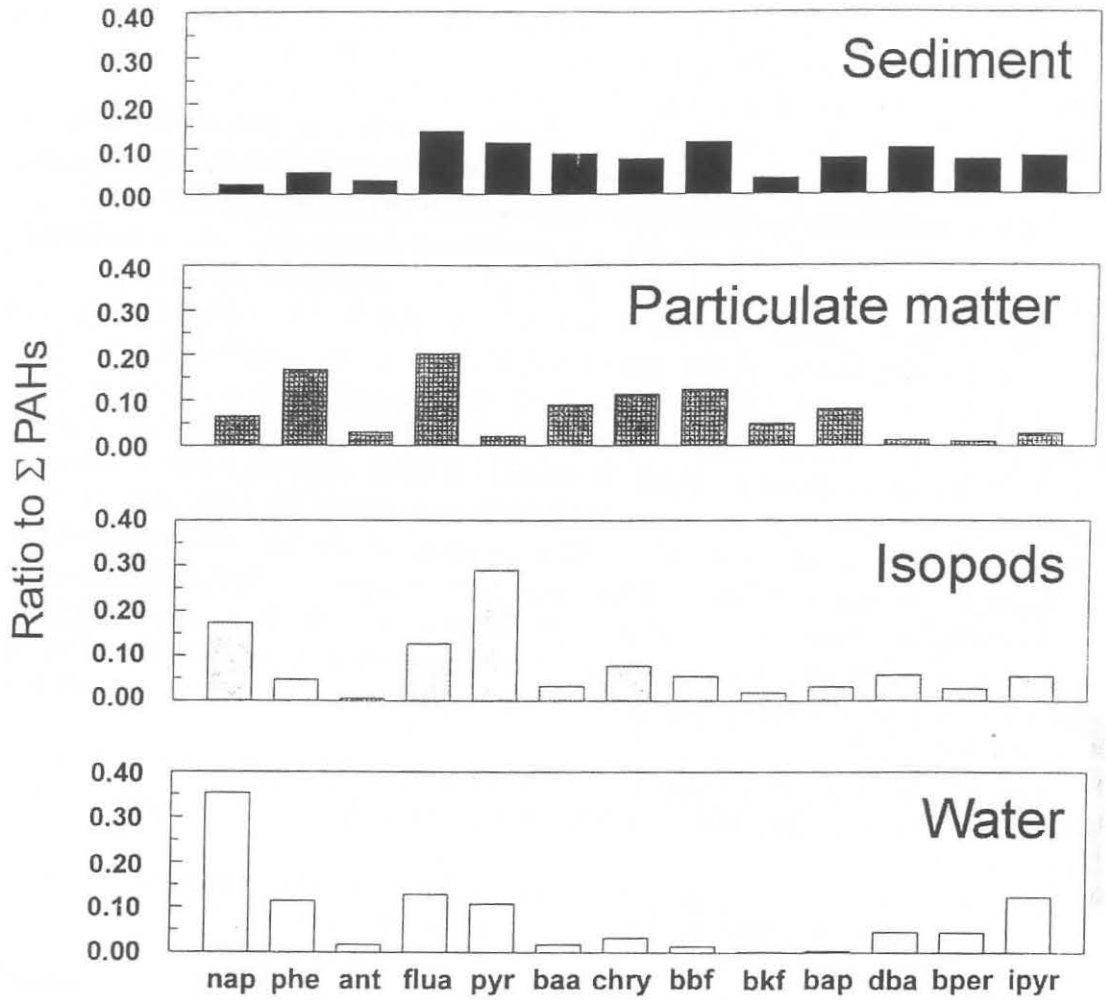

Fig. 2. Patterns of relative concentrations (ratio to ΣPAHs) in sediment, particulate matter, freshwater isopods and filtered water from Lake Hollandsch Diep (Netherlands, Rhine-Meuse estuary)

species seemed limited. Therefore, the preferential bioaccumulation of some PAHs in isopods may be related to the relatively high water solubility of these homologues.

14.2.2.2 *Equilibrium Partitioning Theory*

Most models describing the distribution of compounds in the environment are based on the equilibrium partitioning theory [205, 332]. The concept of equilibrium partitioning assumes that partitioning parameters can be related to compound-specific parameters (hydrophobicity), organism lipid content, and to sediment and water characteristics (e.g. sediment organic carbon content, suspended solids, dissolved organic carbon) [71, 240, 281, 322]. Several authors have demonstrated that the equilibrium partitioning concept, which was originally developed for aquatic laboratory studies, can be successfully applied to

describe the bioaccumulation of contaminants for some terrestrial organisms [46, 48, 326, 327]. In the equilibrium partitioning theory, equilibrium is usually assumed in all biotic and abiotic compartments considered, and concentrations in one compartment can easily be extrapolated to other compartments using partitioning constants. The applicability of equilibrium partitioning theory in field studies on PAHs seems limited due to the influence of non-equilibrium conditions, transformation reactions (photodegradation, biotransformation), the significance of dietary uptake and biotransformation in many species, and to the increased binding of PAHs in aged soils or sediments [23, 168, 320].

In the framework of the equilibrium partitioning concept, biota to soil/sediment accumulation factors (BSAF), normalized to organism lipid content and sediment-organic carbon, are predicted to be similar for most organisms and to be almost independent of the hydrophobicity of the compound [48, 49, 200]. According to Lee et al. [180] the BSAF for most compounds is expected to be approximately 1.7. In an extension of this concept Thomann et al. [308] included the influence of dietary uptake on BSAFs. In their analysis, contributions to BSAF from aqueous and dietary uptake were assumed to be dependent of K_{ow}. The net effect resulted in predicted BSAF values around 0.8–1.0 for compounds with log K_{ow} between 2 and 5, and around 1–10 for more hydrophobic compounds (log K_{ow} between 5 and 8). For compounds which are not subject to extensive metabolism (such as some PCB congeners), good agreement with equilibrium partitioning predictions have been found for various organisms [22, 118, 200, 254].

Several studies in which (lipid, organic carbon normalized) BSAFs of PAHs in invertebrates have been determined are summarized in Table 1. Although the

Table 1. Reported biota to soil/sediment accumulation factors (BSAFs) of PAHs in invertebrates (organism-lipid and sediment/soil-organic carbon normalized)

Species	Habitat[a]	BSAF	No. of PAHs	Type of study[b]	Reference
Mollusks					
Macoma baltica	m	0.5–2.2		f	[90]
		0.2–0.8		l	[88]
Macoma nasuta	m	0.2–1.0		l	[85]
Polychaetes/oligochaetes					
Nereis succinea	m	0.4–1.8		f	[90]
Armandia brevis	m	0.2–1.0	10	l	[211]
Nereis virens	m	0.02–0.07		l	[164]
Lumbricus rubellus	t	0.3–0.9	8	f	[320]
Crustaceans					
Porcellio scaber	t	0.01–0.2	8	f	[320]
Oniscus asellus	t	0.02–0.3	8	f	[320]
Philoscia muscorum	t	0.01–0.5	8	f	[320]
Asellus aquaticus	f	0.1–5	13	f	[54]

[a] marine (m), terrestrial (t), freshwater (f).
[b] field study (f), laboratory study with spiked or field sediments (l).

synopsis is not comprehensive, the BSAF values reported for many species are slightly below predictions from the equilibrium partitioning theory [180]. Several species have extremely low BSAFs, e.g. the polychaete *Nereis virens* and terrestrial isopods, and this has been attributed to biotransformation [164, 320]. In biota-sediment partitioning studies with fish, summarized by McElroy [208] and Meador et al. [212], data have usually been expressed on a dry- or fresh-weight basis without incorporating additional sediment-organic carbon and organism-lipid data. Using average correction factors, the fish data are usually below or similar to the BSAFs reported for metabolizing invertebrates.

In the comparative study of Van Brummelen et al. [320] on soil invertebrates, the BSAF to K_{ow} relationships of isopods differed markedly from that of the annelid *Lumbricus rubellus* (Fig. 3). Marked differences were also observed in concentrations and patterns of PAHs in the different micro-habitats of the topsoil, with concentrations and dominance of high molecular weight PAHs increasing with depth in the order litter layer < fragmentation layer < humic layer. However, this could not explain the differences observed between the patterns in isopods and earthworms. Only the pattern for *Lumbricus* seems to be in agreement with the concept of equilibrium partitioning. For the isopods a strong double-logarithmic negative relationship was found between BSAF and K_{ow} of individual PAHs. The deviation from equilibrium partitioning theory in isopods was attributed to either the dominance of non-equilibrium conditions in the litter-layer, or the absence of skin absorption due to a lack of pore water or impermeability of exoskeleton, and/or to the presence of biotransformation. In the terrestrial environment, the applicability of the equilibrium partitioning concept seems to be limited to species that do not extensively metabolize PAHs and that live in close contact with the soil-pore water system.

14.2.2.3 *Aging*

Prolonged contact times between contaminants and sediment or soil may result in stronger binding and reduced bioavailability that cannot be explained by the time-independent sorption coefficients in the concept of the equilibrium partitioning theory [23, 170]. This process, usually described as 'aging', has been observed in chronic sediment and soil toxicity studies. In spiked sediment experiments with amphipods and midge larvae, reductions of bioaccumulation with a factor 1.4–6 have been observed, after 60–180 days [110, 170]. Varanasi et al. [342] observed 4–6 times lower accumulation of PAHs in bivalves and clams exposed to aged sediments compared to values observed for recently deposited sediments. Compared to spiked soil experiments, Belfroid et al. [21] observed 2–30 times lower bioaccumulation in terrestrial annelids exposed to sediments with a 20–30-year-old contamination of chlorinated monoaromatic compounds. The reduced bioavailability of contaminants in aged sediments is usually attributed to biphasic sorption processes involving both slowly and rapidly exchanging compartments [50, 243, 357]. The decreased desorption with time that results in increasing sediment-water distribution ra-

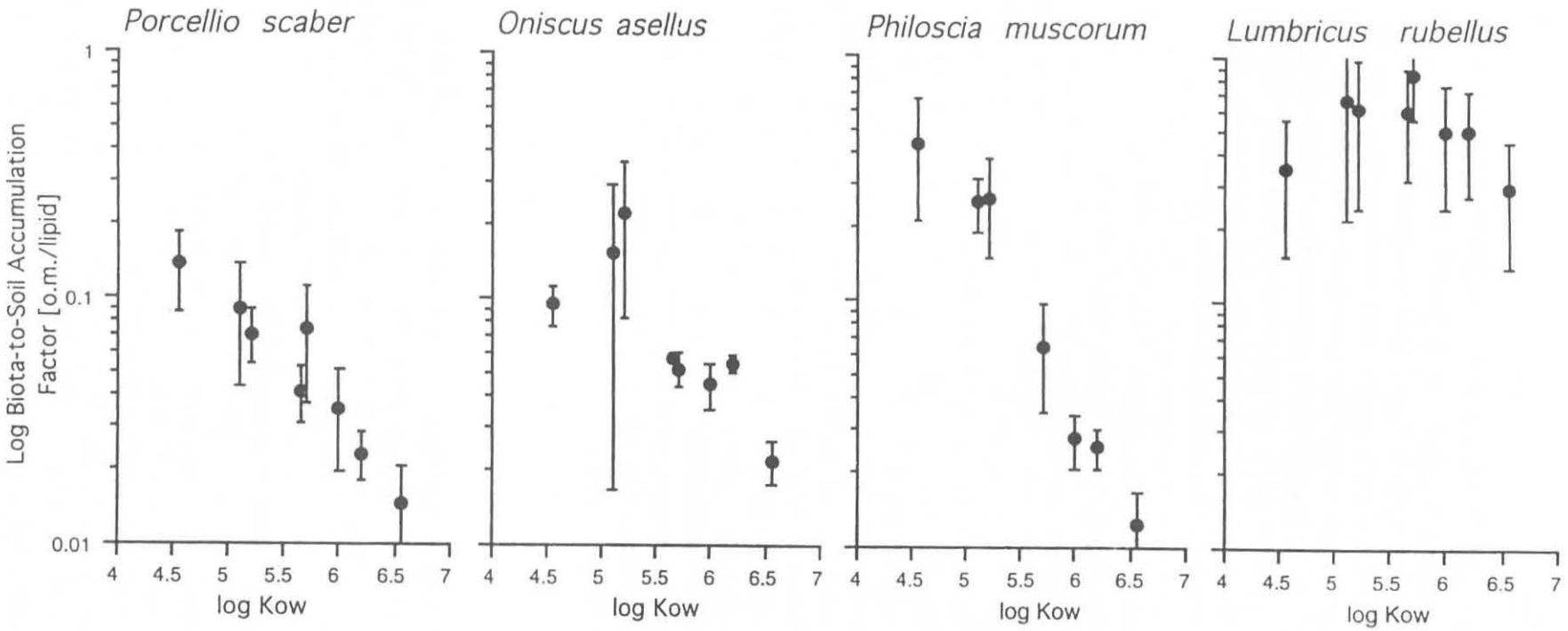

Fig. 3. Biota-to-soil accumulation factors of PAHs in four terrestrial invertebrates in the vicinity of a steelworks and coke-oven complex in the Netherlands [320]

tios is explained by an increase with time of the fraction of contaminants present in the slowly exchanging compartment. This is consistent with organic carbon adsorption coefficients reported for PAHs in freshwater field sediments [54, 153], which are more than one order of magnitude higher compared to values which are commonly used as benchmarks in equilibrium partitioning theory, derived by Karickhoff et al. [151] from laboratory studies with relatively short equilibrium times (< 2 days). The effect of aging may have significant implications for remedial strategies for contaminated soils, harbour dredgings and sediments.

In summary, the bioavailability of PAHs is determined by both biotic and abiotic factors, which at present cannot be rationalized solely on the basis of the equilibrium partitioning theory. Major determinants of bioavailability seem to be:

- the content and composition of organic carbon in soil, sediment, particulate matter and dissolved organic carbon;
- the pre-exposure contact time of contaminants and substrates;
- the heterogeneity or stratification in terrestrial soils;
- feeding behaviour and ecology, which influence exposure; and
- ecophysiological and anatomical characteristics, which determine the mechanisms and kinetics of uptake, biotransformation and elimination.

14.2.3 Toxicokinetic Processes

In terrestrial invertebrates, the uptake of PAHs usually originates from food items, or from epidermal contact with interstitial water. Elimination of accumulated PAHs may be the result of biotransformation and redistribution in the gastro-intestinal tract of parent compounds and biotransformation products. In aquatic environments, aqueous uptake of PAHs (bioconcentration) seems to be dominant in most invertebrates and fish. In several benthic invertebrates significant dietary uptake (bioaccumulation) has been observed. Following uptake, PAHs may be subject to biotransformation, to internal distribution among tissues and organs, and to elimination of parent compounds or biotransformation products. The delicate balance between these processes determines the net bioaccumulation of PAHs. In particular, the ability of biotransformation of PAHs is of prime importance in explaining variability in tissue residues among different species in a specific habitat. A basic understanding of inter-species variation in metabolic capacity and toxicokinetics is required for a proper evaluation of data from field and laboratory studies.

Various mathematical models (rate constant-based, clearance-based, fugacity-based, physiological, pharmacokinetic-based) have been applied to bioconcentration and bioaccumulation studies; for a discussion see [16, 170, 293, 294]. In a relatively simple first-order one compartment model, the bioaccumulation from aqueous and dietary sources can be described by the following differential equation:

$$dC_{org}/dt = k_u \cdot C_w + a \cdot R \cdot C_f - (k_e + k_m) \cdot C_{org} \quad (1)$$

in which:
C_{org} concentration in target organism (µg/kg)
C_w concentrations in (pore)water (µg/l)
C_f concentration in food (µg/kg)
k_e removal rate constant by elimination (e.g. day^{-1}).
k_m removal rate constant by biotransformation to metabolites (e.g. day^{-1})
R consumption rate (food amount per unit body weight per time-unit)
a assimilation efficiency (dimensionless)
t time (e.g. days)

Depending on the experimental design or the time-variability of the exposure concentrations, various analytical solutions of Eq. (1) can be used for the description of the bioaccumulation patterns and parameter estimation of rate constants and assimilation efficiency. In other cases numerical solutions are required [41]. In aquatic studies, the dietary component is usually omitted from the analysis, whereas in terrestrial studies the aqueous component is usually not considered. When metabolism is constitutive (i.e. independent of C_{org}), k_e and k_m are usually combined, sometimes with a growth-dilution parameters [294].

Bioconcentration factors ($BCF = C_{org}/C_w$) and bioaccumulation factors ($BAF = C_{org}/C_f$) can be derived from measured concentrations (apparent and often time-dependent BCFs or BAFs), or calculated from rate constants and assimilation efficiency. Such rate constant-based BCFs and BAFs are approximations to theoretical steady state conditions. Due to limitations of the experimental design [62, De Maagd, Chap. 15] the rate constant of biotransformation cannot usually be determined separately.

A summary of BCFs of different PAHs in primarily aquatic invertebrates is presented in Table 2. Extensive studies have been conducted with freshwater and marine invertebrates but to our knowledge the number of studies with PAHs in terrestrial invertebrates is limited to the studies of Faber and Heijmans [80], Van Straalen et al. [337] and Van Brummelen et al. [318, 320, 321].

The highest wet weight BCFs indicated in Table 2 have been reported for the freshwater amphipod *Diporeia* spp.[166] and the freshwater isopod *Asellus aquaticus* [329]. *Diporeia* spp. inhabit the deep sediments of the North American Great Lakes [91, 166], whereas *Asellus aquaticus* is a littoral detritivore commonly found in shallow waters. In both species the role of biotransformation seems to be limited [166, 329]. A comparison of BCFs on a lipid basis is not possible since lipid contents have not been reported consistently. The bioaccumulation of high concentrations of PAHs in other aquatic isopods has been reported previously for the marine *Lygia* spp. from an atoll in the Pacific Ocean [233]. In terrestrial crustaceans, low bioaccumulation of benzo[*a*]pyrene and other PAHs was observed in both field and experimental work [80, 318, 320, 321, 337]. In an experimental study, it was demonstrated by Van Brummelen and Van Straalen [318] that benzo[*a*]pyrene added to the food was available to the isopod *Porcellio scaber*, but that a high elimination rate was responsible for low residues. Formation of hydroxylated pyrene metabolites in *Porcellio scaber* was subsequently confirmed by Stroomberg et al. [304]. Apparently, the toxico-

Table 2. Experimental wet weight bioconcentration or bioaccumulation factors (BCF, BMF) and first-order one-compartment toxicokinetic parameters (k_1, k_2, $t_{0.5}$) of PAHs in aquatic and terrestrial invertebrates

Species/compound	Lipid %	Log BCF [a] L kg^{-1}	k_1 L kg^{-1} day^{-1}	k_2 day^{-1}	Ref.
Freshwater invertebrates					
Diporeia hoyi [b]	4–12				[166]
– anthracene		(4.6)	3144	0.08	
– phenanthrene		(4.4)	3096	0.11	
– pyrene		(5.2)	4776	0.03	
– benzo[*a*]anthracene		(4.8)	3312	0.05	
– benzo[*a*]pyrene		(4.9)	2808	0.04	
Hyalella azteca [c]					
– anthracene		3.2	2–10.10^3	2.4–13.6	[74, 174]
Mysis relicta [d]					
– phenanthrene		(3.6)	768	0.28	[92]
– anthracene		(3.6)	1512	0.36	
– benzo[*a*]pyrene		(3.9)	2688	0.31	
Chironomus riparius [e]					
– anthracene		1.6–2.1	41–732	1.7–6.6	[99]
– benzo[*a*]pyrene		2.3	5136	0.5–5.2	[187]
Hexagenia limbata [f]					
– phenanthrene		4.1	14016	1.2	[300]
– benzo[*a*]pyrene		3.9	3768	0.48	
Stylodrilus heringianus [g]					
– anthracene		(3.7)	2112	0.4–0.5	[91]
– phenanthrene		(3.7)	2256	0.4	
– pyrene		(3.8)	2729	0.4	
– benzo[*a*]pyrene		(3.8)	2107	0.3–0.4	
Asellus aquaticus [h]	0.1–0.5				
– anthracene		2.6	890	1.0	[329]
– phenanthrene		3.2	1580	1.2	
– pyrene		4.4	3490	0.82	
– benzo[*a*]pyrene		4.4	2410	0.10	
– benzo[*e*]pyrene		4.6	8310	0.09	
– benzo[*ghi*]perylene		5.7	4030	0.36	
Marine invertebrates [i]					
Mytilus edulis					
– phenanthrene		3.5	502	0.14	[210]
– pyrene		3.7	480	0.10	
– perylene		4.0	182	0.02	
Mya arenaria					
– phenanthrene		2.9	197	0.22	[210]
– pyrene		3.3	264	0.12	
– perylene		3.3	91		
Crangon septemspinosa					
– phenanthrene		3.2	754	0.48	[210]
– pyrene		2.9	470	0.53	
– perylene		2.6	185	0.48	

Table 2 (continued)

Species/compound	Lipid %	Log BCF [a] L kg^{-1}	k_1 L kg^{-1} day^{-1}	k_2 day^{-1}	Ref.
Nereis virens					[210]
– phenanthrene		3.6	264	0.72	
– pyrene		3.1	1286	0.60	
– perylene		2.7	91	0.17	
Fish-species					
Pimephales promelas					
– naphthalene		2.5	1500	5	[62]
– phenanthrene		3.8	2000	0.3	
– anthracene		3.8	2700	0.4	
– fluoranthene		3.5	2700	0.8[j]	
– benz[*a*]anthracene		2.3	2300	11.3[j]	
Poecilia reticulata					
– fluorene		3.4	1440	0.6	[64] [k]
– anthracene		3.9	1840	0.3	
– pyrene		3.7	3232	0.7	
Terrestrial invertebrates					
Porcellio scaber					
– benzo[*a*]pyrene				1.1	[318]

[a] BCFs between brackets were recalculated from source data as ratio of k_1 and k_2.
[b] organism formerly indicated as *Pontaporeia hoyi*, 6 h exposure/14 days elimination studies; average values at 4°C.
[c] BCFs at 8 h; rate constants: range of values for various experimental treatments at 2–25°C
[d] 6 h exposure at 4°C; BCF values calculated from k_1 and k_2.
[e] anthracene: range of results from 4–30 h experiments at 16–30°C; benzo[*a*]pyrene: 16 h experiments, elimination rate constants for biphasic elimination.
[f] 120-day mayfly nymphs, 224 h experiments at 18°C.
[g] 21 h experiments; range of values for various experimental treatments at 4°C. h: 7-day uptake, 14-day elimination experiments.
[i] 4-day uptake, 14-day elimination experiments.
[j] including rate constant for metabolism (fluoranthene 0.1 day^{-1}; benz[*a*]anthracene 11 day^{-1}); 48 h static exposure experiments.
[k] from 48-h uptake experiments.

kinetics of PAHs in isopods from different suborders and from terrestrial and aquatic environments are really not comparable.

The uptake rate constants of different PAHs (Table 2) for freshwater invertebrates range from 770 (phenanthrene in *Mysis*) to 14000 (phenanthrene in *Hexagenia*), but most of the values seem to be in the range 1000–4000 (l kg^{-1} d^{-1}), and are comparable to the values found in short term (48 h) studies with PAHs in fish [62–64]. In the comprehensive review of Opperhuizen et al. [247], similar values were reported for polyhalogenated hydrocarbons in fish. The uptake rate constant values of PAHs in marine invertebrates reported by McLeese and Burridge [210] seem to be lower (91–1286) compared to those for freshwater invertebrates. No systematic differences seem to be present in uptake

clearance rate constants between different PAHs, and this is in agreement with observations for other groups of compounds [112, 247].

The elimination rates of individual PAHs (Table 2) vary considerably among species. The highest values (1–13 d^{-1}) are noted for invertebrates and fish species in which biotransformation is dominant. In some non-metabolizing species, such as *Diporeia* spp and *Asellus aquaticus,* the elimination rate constants decrease with increasing hydrophobicity [166, 329]. This is consistent with observations for other persistent neutral organic compounds in aquatic organisms. Elimination rate constants usually decrease with K_{ow} and double logarithmic regression relationships have been reported for different classes of compounds in various groups of organisms [112, 157, 166, 247, 293]. Landrum [166] conducted comprehensive studies on factors that determine elimination rate constants of PAHs in the amphipod *Diporeia* spp, and demonstrated dependence on lipophilicity, season, temperature, age, body weight, and lipid content.

14.2.3.1 *Relationships with K_{ow}*

Bioconcentration factors and first-order elimination rate constants for persistent neutral organic compounds in aquatic organisms usually exhibit strong (double logarithmic) regression relationships with K_{ow}[46, 112, 198, 206, 229, 246, 345]. The relationship proposed by Mackay [198] for (wet weight) BCFs of compounds in fish is commonly considered a benchmark for the equilibrium partitioning model [46, 205, 206]. In Table 3 several QSARs for K_{ow} and BCFs of PAHs in various organisms have been presented. Although the conditions of the different experiments varied considerably, there is a remarkable agreement among the slopes of the regression coefficients of the relationships for the dif-

Table 3. Linear regression relationships between wet weight bioconcentration factors (BCFs) of PAHs and K_{ow} · (Log BCF = a + b · Log K_{ow}) for PAHs in aquatic invertebrates[a]

Species/taxon	a	b	R^2	s_y	N	n	Ref.
Mytilus edulis	– 2.4	1.1 ± 0.3	0.88		11	5	[73]
Daphnia pulex	– 0.4	0.75	0.85		7	6	[292]
Daphnia magna	– 0.05	0.66	0.65		6	3	[75]
Diporeia spp.	1.8 ± 0.2	0.65 ± 0.05	0.99	0.1	4	4	[166][d]
Asellus aquaticus	– 2.2 ± 0.9	1.1 ± 0.1	0.96	0.2	5[c]	5	[329]
Mollusks	– 1.23	0.84	0.69		34	1	[112]
Daphnids	– 1.32	0.90	0.92		22	8	[112]
Fish spp.[b]	– 1.32	1.00	0.95	0.25	36	9	[198]

[a] Symbols: a = regression constant; b = regression slope; R^2 = coefficient of determination; s_y = standard error of estimate; N = total number of compounds included in equation; n = number of parent PAHs included in equation.

[b] QSAR relationship of Mackay [198] for different fish species included in equation.

[c] Benzo[ghi]perylene was excluded.

[d] Calculated from reported data.

ferent taxa. The differences between organisms in the regression probably reflect primarily variations in lipid content. The QSAR for fish species in the study of Mackay [198], with an estimated regression constant of −1.32, is based on different species of fish, with a mean lipid content of around 5% [206]. The extremely high bioconcentration in *Diporeia* spp. was attributed by Landrum [166] to the high lipid content (4–12%) of this profundal freshwater amphipod. The mean lipid contents of the daphnids and molluscs in the study of Hawker and Connell [112] were 4% and 1–2% respectively [47]. Although the freshwater isopod *Asellus aquaticus* had a relatively low lipid content (0.34 ± 0.07%), it accumulated high concentrations of PAHs [329].

Lower bioconcentration of higher PAHs has been observed in various laboratory and field studies [62, 63, 212, 329]. For many other hydrophobic compounds with log K_{ow} exceeding 6–7, this phenomenon has been reported and attributed to various factors, including the following:

- reduced membrane permeability in relation to molecular size [247]
- lipid solubility and limited predictive capacity of K_{ow} [40, 64]
- biotransformation [246]
- insufficient duration of exposure in relation to time required to attain effective equilibrium conditions [46, 115]
- limited bioavailability
- combinations of these factors [196]

Connell and Hawker [47] reanalyzed published bioconcentration data for fish and proposed a 4th-order polynomial relationship with a maximum wet weight log BCF value of 4.6 for compounds with a log K_{ow} value of 6.7. Related parabolic (2nd order) and bilinear relationships have been proposed by Bintein et al.[26].

14.2.3.2 *Role of Dietary Uptake*

The role of dietary uptake (bioaccumulation) in aquatic studies seems to have been underestimated in the past, and this route of uptake is usually not considered in equilibrium partitioning based risk assessment models. Although for many invertebrates and compounds, uptake from pore water or the water column is the dominant route of uptake [22, 23, 244, 279], several epibenthic or infaunal species of invertebrates can efficiently accumulate PAHs from ingested sediments or detritus [308]. In the studies of McLeese and Burridge [210] shrimps and polychaetes accumulated PAHs from ingested sediments. Several studies in which the gastro-intestinal assimilation of PAHs has been estimated are summarized in Table 4. The assimilation efficiency for dietary uptake of PAHs may range from 4–60%. In the study of Weston [356] with *Abarenicola pacifica*, the assimilation efficiency of benzo[*a*]pyrene seemed to decrease with increasing concentration. An overview of dietary uptake in marine organisms has been given by Meador et al. [212].

Although the assimilation efficiency of PAHs seems to decrease with K_{ow} [212], the relative importance of dietary uptake may increase for more hydro-

Table 4. Dietary uptake – Assimilation efficiencies reported in the literature

Species	Compound	Assimilation efficiency	Reference
Terrestrial invertebrates			
– *Porcellio scaber*	benzo[*a*]pyrene	20–40%	[318]
Aquatic invertebrates			
– *Calanus helgolandicus*	naphthalene	< 60%	[111]
– *Callinectes sapidus*	benzo[*a*]pyrene and other PAHs	2–10%	[183]
– *Abarenicola pacifica*	benzo[*a*]pyrene	1–99%[a]	[356]
– *Diporeia* spp.	benzo[*a*]pyrene	46–60%	[170]
		6–33%	[110]
– *Hexagenia limbata*	benzo[*a*]pyrene	13–20%	[172][b]
Fish			
– rainbow trout	various PAHs	2–32%	[234]
	fluorene	14%	[235]
	phenanthrene	4%	
	anthracene, and higher PAHs	< 1%	

[a] depending on dose and TOC of spiked sediments, see figure in Meador et al. [212].
[b] calculated from data.

phobic compounds, since abiotic partitioning may lead to severe reductions in bioavailability [308].

14.2.4 Biotransformation

In aquatic ecosystems, PAHs are present predominantly in the abiotic compartments (water, suspended matter, sediments) and the lower trophic levels (plankton, macrophytes, invertebrates). Cytochrome P450-mediated biotransformation of PAHs is well established in mammals, birds and many fish species [230, 343, 351, 352]. Most invertebrates have a less well developed MFO-system [139, 334, 342]. In the past, biotransformation of PAHs by invertebrates was often assumed to be of limited importance. However, from a number of studies during the last decade, evidence has emerged for the existence of large differences in rates of biotransformation among and within invertebrate taxa [74, 139, 208, 210, 231, 342]. For several bivalve molluscs and oligochaete species, biotransformation of PAHs was observed to be low [91, 139, 192, 296]. Within the phylum of aquatic arthropods, large differences in biotransformation rates appear to exist, even between closely related species such as amphipods [166, 174, 266]. Similar differences in biotransformation rates are present within other groups of phylogenetically related species, such as crustaceans [92], freshwater insects [99, 300] and marine polychaetes [208]. Due to these large species-specific differences in biotransformation of PAHs, care should be taken in the interpretation of tissue residues of PAHs in organisms from biomonitoring programs.

Detoxification systems like the MFO system are thought to have developed a higher activity in terrestrial invertebrates than in aquatic invertebrates. The limited availability of water in the terrestrial environment restricts the elimination of lipophilic parent compounds and increases the need to convert xenobiotics into water soluble compound which can be excreted with, for example, urine [350, 351]. The presence of numerous plant toxins in the diet of many terrestrial animals has also been suggested as a selective force for efficient detoxification enzymes [32, 162, 225]. Differences in the activity of detoxification enzymes between aquatic and terrestrial species have indeed been reported [283, 302, 351], and may form an explanation for the relatively high accumulation of PAHs by many aquatic isopods compared to terrestrial isopods.

14.2.5 *Trophic Transfer*

Due to the biotransformation of PAHs in vertebrates and some invertebrates, food chain transfer and biomagnification of PAHs do not appear to exist [33, 43, 230]. Although some primary consumers and detritivores may accumulate high levels of PAHs [72, 166, 197, 233, 292, 329, 337], predators usually contain low levels [33, 114–116, 186, 234].

In Fig. 4 an example is presented of benzo[*a*]pyrene concentrations in aquatic organisms from various interconnected waters in the Rhine-Meuse estuary based on [330, 331] and unpublished results. The absence of biomagnification is clearly illustrated. The qualitative comparison of lipid based concentrations

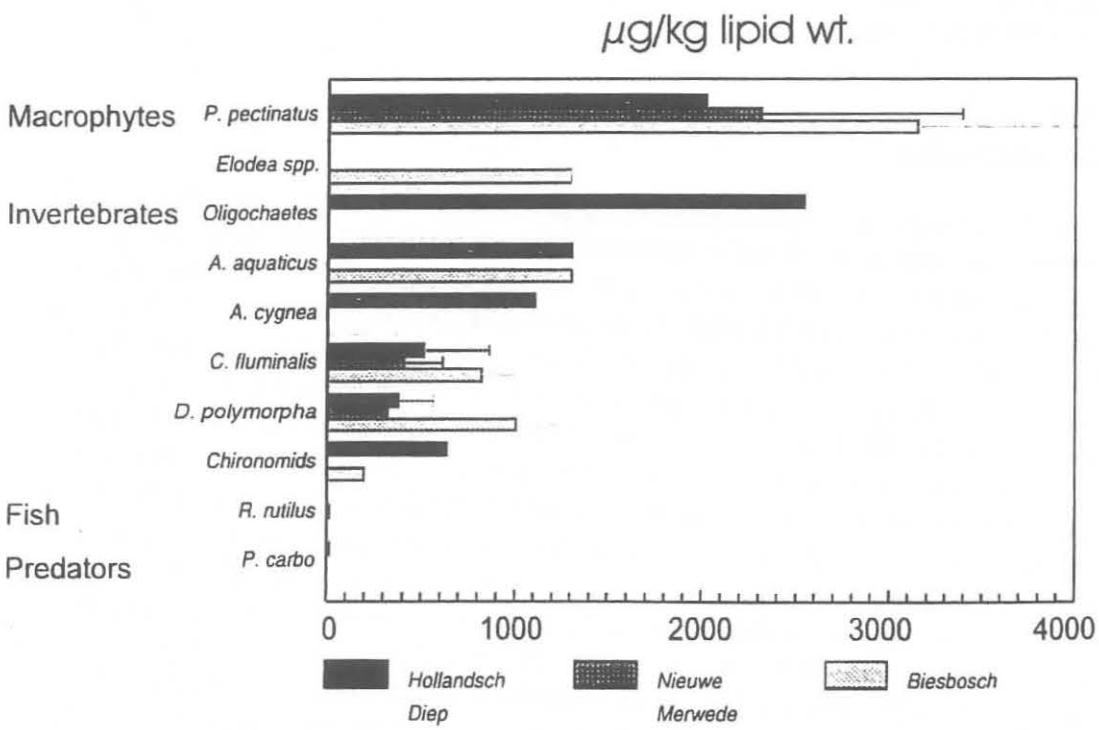

Fig. 4. Benzo[*a*]pyrene (µg kg^{-1} lipid wt.) in organisms from adjacent waters in the Rhine-Meuse estuary

shows that the highest concentrations were encountered in aquatic plants (pondweed *Potomogeton pectinatus*; waterweed *Elodea* spp.), oligochaetes, isopods and freshwater clams (*Anodonta cygnea*). Lower concentrations were observed in other molluscs (*Dreissena polymorpha*, *Corbicula* spp.) and in chironomids. Concentrations close to detection limits were observed in whole body homogenates of roach (*Rutilus rutilus*) and in the liver of seven-week-old cormorant chicks from the Biesbosch colony, feeding on roach and other cyprinids from this area. Due to variations in organism lipid content, a different ranking is seen when data are expressed on a wet weight basis. Wet weight concentrations decreased in the order: isopods > oligochaetes > aquatic plants > *Anodonta* > other mollusks and chironomids > fish. For other PAHs, similar patterns of differences between species were observed. The process of decreasing concentrations with rising trophic level, that has also been observed for some trace metals, has been termed 'biominification' [38].

14.2.6 Conclusions

The fraction of PAHs that is available for uptake by organisms varies between species, depending on the relative significance of different uptake pathways. Therefore, bioavailability always has to be related to a (group of) species with specifically defined route(s) of uptake.

The best way to determine PAH bioavailability is to determine the uptake rate parameters in a kinetic study. This is however quite laborious and bioavailability is usually assessed on the basis of tissue residues. It is obvious that such an assessment can only be made if sufficient knowledge is available on kinetics and mechanisms of uptake, biotransformation and elimination, since large variations exist among different taxa of the animal kingdom. Many other biological factors (e.g. life stage, body weight, lipid weight, moulting, feeding habits, reproduction) may contribute to the within-species variability of toxicokinetics of PAHs.

Bioavailability of PAHs seems to be determined both by biotic and abiotic factors, which cannot be satisfactorily explained within the existing equilibrium partitioning theory. Major determinants of bioavailability are: the content and composition of organic carbon in soil or sediment, particulate matter and dissolved organic carbon; the pre-exposure contact time of contaminants and substrates; heterogeneity or stratification in terrestrial soils; and the route of exposure, (eco)physiological and anatomical characteristics, feeding behaviour and ecology.

14.3 Mechanisms of PAH Toxicity

It is often stated that body residues reflect the internal concentration close to the site of toxic action, but what toxic action is meant here? This toxicity mechanism would be responsible for the occurrence of a variety of biological processes that include the following illustrative examples:

- benzo[*a*]pyrene affected the development of flatfish and sea urchin [130, 131]
- PAHs affect the immune-response of mussels [45, 104]

- both benzo[*a*]pyrene and fluoranthene reduced the feeding rate of *Mytilus edulis* [78]
- naphthalene affected the oxygen consumption by crustaceans and insects [52, 58]
- negative effects of PAHs on growth, reproduction and survival have been documented by many authors (e.g. [96, 108, 291, 312, 316])

These examples of biological effects of PAH exposure are probably the result of a number of different mechanisms of toxicity. This section describes the mechanisms of toxicity that have been postulated.

14.3.1 Nonpolar Narcosis

Nonpolar narcosis or baseline toxicity is an aspecific mode of toxicity of non-reactive, non-electrolyte organics and is thought to result from the accumulation of the toxicant in the biological membrane. The term "non-reactive" is of course relative, since almost no compounds are completely unreactive under all conditions. The structure of the membrane is disturbed by the presence of the toxicants, and essential membrane bound processes such as osmoregulation and neurotransmission are affected (see reviews by Lipnick [190] and Van Wezel and Opperhuizen [339]). The disturbance of the membrane structure is caused by the physical presence of toxicant molecules and less so by the chemical nature of these non-reactive compounds. Narcosis can develop relatively quickly during short term exposure and is a phenomenon which is typically observed in acute toxicity tests. In fish, narcosis or anesthesia results in a specific characteristic behaviour: initial excitement, disoriented behaviour followed by immobility and death.

The non-specific nature of this mode of toxicity offers opportunities for scientific generalization. Successful attempts have been made to derive QSARs and predict the toxicity of narcotic chemicals on the basis of K_{ow}, that significantly determines the accumulation of a compound in biological membranes (e.g. [156, 333, 344]).

According to the classification scheme for chemicals which was developed by Verhaar et al. [346], PAHs are categorized as inert chemicals which exert narcosis. Chemicals with a more specific mode of action are more toxic and for this reason narcosis is also called baseline toxicity. The other toxicity mechanisms of PAHs that are summarized below all imply that PAHs can be much more reactive, and that at least under certain conditions PAHs will have a higher toxicity than the baseline toxicity.

14.3.2 Phototoxicity

The exposure to UV light may increase the toxicity of certain PAHs. This was observed for a variety of organisms including the following:

- *Photobacterium phosphoreum* [11]
- the alga *Selenastrum capricornutum* [44, 94]

- the plant species *Lemna gibba* [133, 268]
- the crustaceans *Daphnia magna* [123], *D. pulex* [5] and *Artemia salina* [146]
- the insect *Aedes aegypti* [145, 146]
- the fish species *Lepomis macrochirus* [207, 249] and *Pimephales promelas* [145, 248]
- the amphibian *Rana pipiens* [146, 147]

Phototoxicity has also been observed in field studies with the oligochaete *Lumbricus variegatus* [223]. Phototoxicity of PAHs has recently been reviewed by Arfsten et al. [12].

The light-induced formation of free radicals and subsequent damage to a variety of macromolecules is believed to be responsible for the increased toxicity of PAHs in the presence of UV light [169]. This type of toxicity can develop relatively quickly and can be observed in acute toxicity experiments. The chemical mechanism is not yet fully understood and may involve formation of PAH radicals and/or oxygen radicals. In the review by Landrum et al. [169] a number of hypotheses are summarized and the reader is referred to this publication for details.

Phototoxicity is a specific toxicity mechanism since the presence of UV radiation causes only some PAHs to have a higher toxicity than would be expected on the basis of nonpolar narcotic action. The relative phototoxicity of 20 PAHs at equimolar body concentrations has been studied for the crustacean *Daphnia magna* and a curve-linear model to predict photo induced toxicity was developed by Newsted and Giesy [232]. Anthracene, pyrene, benzo[*a*]pyrene and dibenzo[*a,h*]anthracene were among the most phototoxic compounds, whereas phenanthrene, fluorene and triphenylene did not elicit phototoxicity (Table 5). The model of Newsted and Giesy [232] was refined by Mekenyan et al. [215], who demonstrated that one single structure related molecular descriptor could be used to explain the observed phototoxicity of PAHs. This descriptor, the HOMO-LUMO gap, defines the energy necessary to excite an electron from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). With increasing HOMO-LUMO gap the light absorption shifts to a shorter wavelength (higher energy) and the potential phototoxicity of the compound increases. With decreasing wavelength, however, the intensity in sunlight slowly decreases to zero. Some PAHs (e.g. phenanthrene) may thus have a high HOMO-LUMO gap but it may not be phototoxic as the short wavelength light necessary to excite the molecule is not available in sunlight. See Mekenyan et al. [215] for further details.

Differences in the phototoxicity of PAHs among organisms may be caused by a number of factors. These include:

(1) the quality and quantity of sunlight penetrating into the organism and this may vary with the habitat of the organism and with light absorbency due to pigmentation or shielding (e.g. bivalves)
(2) the internal exposure concentrations that may differ widely between species; the factors influencing these residue levels have been discussed in an earlier section
(3) organisms may differ in their protective mechanisms against free radicals [17]

Table 5. Phototoxicity to *Daphnia magna* [215, 232] and carcinogenicity to humans [136] of selected polycyclic aromatic hydrocarbons

Compound	Photoxicity *Daphnia magna* [a]	Carcinogenicity to humans[b]	CAS number
Anthracene	++	3	120-12-7
Benz[*a*]anthracene	++	2A	56-55-3
Benzo[*a*]fluorene	+	3	238-84-6
Benzo[*a*]pyrene	++	2A	50-32-8
Benzo[*b*]fluoranthene		2B	205-99-2
Benzo[*b*]fluorene	+	3	243-17-4
Benzo[*c*]fluorene		3	205-12-9
Benzo[*c*]phenanthrene		3	195-18-7
Benzo[*e*]pyrene	++	3	192-97-2
Benzo[*g,h,i*]fluoranthene		3	203-12-3
Benzo[*g,h,i*]perylene	++	3	191-24-2
Benzo[*j*]fluoranthene		2B	205-82-3
Benzo[*k*]fluoranthene	++	2B	207-08-9
Chrysene	+	3	218-01-9
Corenene	(0)	3	191-07-1
Dibenz[*a,h*]anthracene	++	2A	53-70-3
Dibenzo[*a,c*]anthracene	(++)	3	215-58-7
Dibenzo[*a,j*]anthracene	(++)	3	224-41-9
Dibenzo[*h,rst*]pentaphene		3	192-47-2
Dibenzo[*a,e*]pyrene		2B	192-65-4
Dibenzo[*a,h*]pyrene		2B	189-64-0
Dibenzo[*a,i*]pyrene		2B	189-55-9
Dibenzo[*a,l*]pyrene		2B	193-30-0
Fluoranthene	++	3	206-44-0
Fluorene	0	3	86-73-7
Indeno[1, 2, 3-*cd*]pyrene		2B	193-39-5
Naphthalene	(0)		91-20-3
Perylene	++	3	198-55-0
Phenanthene	0	3	85-01-8
Pyrene	++	3	129-00-0
Triphenylene	0	3	217-59-4

[a] experimental and model-derived (in brackets) values [215, 232]
0 not phototoxic
+ moderately toxic
++ very toxic.

[b] IARC classification [136]
1 carcinogenic to humans
2A probably carcinogenic to humans
2B possibly carcinogenic to humans
3 unclassifiable as to carcinogenicity to humans
4 probably not carcinogenic to humans.

14.3.3
Biochemical Activation and Subsequent Adduct Formation

This is probably the best-known toxicity mechanism of PAHs. During enzymatic transformation certain PAHs are transformed into highly reactive compounds which may form covalent bonds with macromolecules such as protein and DNA, the adducts. The textbook-example is the activation of benzo[*a*]-pyrene to its 7,8-diol-9,10-epoxide and subsequent binding to the DNA base guanine [311]. DNA adducts may give rise to mutations and this may result in carcinogenesis and teratogenesis, typical parameters of chronic toxicity experiments which take a long time to develop. The various mechanisms for activation of PAHs have been discussed by Cavalieri and Rogan (Chap. 11).

The Mixed Function Oxygenase (MFO) system is thought to be responsible for the initial introduction of oxygen into PAHs. This enzymatic system is located in the smooth endoplasmatic reticulum and contains cytochrome P-450 isozymes and cytochrome P-450 NADPH reductase. A number of reviews has been published on this enzyme system and its occurrence in different phyla [32, 35, 121, 140, 193, 271, 297, 343, 350–352].

Covalent binding of benzo[*a*]pyrene metabolites to protein and DNA has been observed in a variety of organisms, e.g. the mollusc *Mytilus edulis* [201], the crustaceans *Carcinus maenas* [201] and *Homarus americanus* [141], the insect *Spodoptera eridania* [8] the echinoderm *Asterias rubens* [201], and a variety of fish species, e.g. [286, 340, 343]. PAH metabolism is discussed in greater detail for mammals by Cavalieri and Rogan (Chap. 11) and for aquatic organisms by de Maagd and Vethaak (Chap. 15).

In many organisms elevated environmental levels of PAHs and other toxicants (e.g. PCBs, PCDDs and PCDFs) cause an increase in the activity of the MFO system (induction). This will result in an increase of metabolic activation and a subsequent rise in PAH adduct formation. The induction will stimulate the elimination of toxicants from the tissue (detoxification), resulting in reduced residue levels of the parent PAH. A nice example of this process was observed by Van der Oost et al. [325] who analyzed eels (*Anguilla anguilla*) from contaminated and clean field sites. At the contaminated sites they found reduced PAH concentrations in the fish, in association with increased 1-OH pyrene excretion and increased DNA adduct levels, compared to reference sites.

In chironomids from contaminated field locations, an increased occurrence of deformities has been observed [67], while an increased incidence of hepatic lesions has been documented for fish [19, 143, 165, 199, 218, 227, 348 and de Maagd and Vethaak, Chap. 15] and beluga whales [59]. In these studies PAHs have been suggested as possible causative agents.

Biochemical activation and subsequent adduct formation is a specific toxicity mechanism for several reasons: (1) only certain PAHs are metabolically activated and are registered as carcinogens, e.g. benzo[*a*]pyrene is generally considered a potent carcinogen, whereas benzo[*e*]pyrene is a typical example of a non- or much less potent carcinogenic PAH; (2) not all organisms are equally equipped with the enzyme systems needed to carry out extensive metabolism of PAHs. Exposed organisms that have a high rate of metabolism of PAHs are

likely to be the victim of this type of toxicity, particularly if the cellular DNA-repair systems are poorly developed.

14.3.4 Disturbance of Hormonal Regulation

Some PAHs are similar in structure to steroid hormones, such as ecdysteroids and estradiols and interference of PAHs with hormone regulation has been suggested by a number of authors e.g. [108, 182, 256, 313, 317]. A direct hormonal effect of PAHs may occur when metabolites of PAHs interact with the hormone receptors. This mimicking of hormones is only likely when the contaminants have sufficient structural similarity with the hormones in question. PAHs would require at least four aromatic rings and metabolization since phenolic or quinone groups are required for interaction with the receptors [70, 144, 224, 261].

The hormonal balance is affected indirectly when elevated levels of PAHs in the environment cause an increase in the activity of the MFO system (induction). The MFO system is involved both in the activation and deactivation of steroid compounds and in the metabolism of a range of xenobiotics [121, 182, 256, 354]. Induction of MFO activity by PAH exposure occurs both in insects [154] and in fish [36], although it is not yet clear whether induction also occurs in molluscs and crustaceans [139, 140, 193]. To our knowledge there is only circumstantial evidence for interference of PAHs with hormonal regulation. Assuming this toxicity mechanism occurs, differences in the potency of PAHs are to be expected that are related to the capacity to induce MFO activity (indirect action) and the structural resemblance of the molecules with steroid hormones (direct action). This type of toxicity will take some time to develop and will require chronic exposure.

14.3.5 Conclusions

There is a theoretical link between residue levels and toxicity since residue levels are assumed to reflect the concentration inside the organism close to the site of toxic action. In the first section dealing with residue levels, it was demonstrated that metabolism of PAHs is a factor which has considerable influence on the residue levels within organisms. Organisms which metabolize PAHs extensively generally have lower residue levels compared to organisms which do not metabolize PAHs, and they will be less susceptible to narcosis and phototoxicity of PAHs since the concentrations at the target site will be lower. At the same time, however, organisms that have a high rate of metabolism of PAHs are likely to be the victim of adduct formation or disturbance of the hormone regulation.

Consequently the relationship between residue levels of PAHs and toxicity is ambiguous: a low residue level in an organism means either that exposure to PAHs is low and that there is little risk, or it may mean that the organism has a very active, possibly induced, MFO system which results in adduct formation or

disturbance of hormone regulation. In that case the low residue levels provide no information on the exposure or risk levels [325]. Clearly species specific information should be used when considering the relationship between residue level of PAHs and the possible risk to the organism.

The limited knowledge of the toxicity mechanisms of PAHs presented here indicates that many PAHs can act through a more specific toxicity mechanism than nonpolar narcosis. Exceptions to this may be PAHs such as naphthalene, phenanthrene and fluorene, which are non-phototoxic, are not considered to be mutagens and do not have a structure similar to hormones. These compounds, which apparently have narcotic action only, are quite abundant in the environment (Simoneit, Chap. 5) but are susceptible to biodegradation (Neilson and Allard, Chap. 10). PAHs with a higher molecular weight generally have longer residence times and have a more specific toxicological nature which may result in adverse effects during chronic exposure.

14.4 Ecotoxicity of PAHs

Results of ecotoxicity tests are often used for risk assessment of substances, in order to estimate "safe" levels for the environment. Especially in the field of aquatic ecotoxicology numerous ecotoxicity tests have been developed (e.g. [239, 241]). In terrestrial ecotoxicology at this moment fewer ecotoxicity tests are available [240, 241] but a lot of information is available on non-standardized tests [336].

Ecotoxicity tests are performed under well-defined laboratory conditions, using standardized water or soil and organisms from laboratory stocks. The toxic substance in question is added in a concentration range to the medium and organisms are added. After a defined time-interval the effect of the different concentrations applied are evaluated. In general, endpoints like survival, growth and reproduction are taken into account as these parameters are considered of importance for population growth.

The ecotoxicity data on PAHs are reviewed (Sect. 14.4.1) and attention is focused on some general patterns in the data. Two specific questions are addressed: (1) is there evidence for specific toxicity mechanisms (Sect. 14.4.2), and (2) is it possible to predict consequences for the population on the basis of these data (Sect. 14.4.3). In Sect. 14.4.4 a comparison of sensitivities of freshwater and marine species is made and Sect. 14.4.5 addresses the use of these data for risk-assessment of PAHs.

14.4.1 Data on PAHs

The toxicity data reviewed here were originally compiled for the development of national ecotoxicological quality objectives in the Netherlands [148, 149], and for this reason only those parameters are taken into account that may affect species at the population level. The toxicity data presented are based on laboratory experiments which compare a decrease in survival, or growth and/or reproduction relative to the control.

Only those studies which meet specific quality criteria were included. A study is considered to be reliable if the design of the experiment is in agreement with internationally accepted guidelines such as the OECD guidelines. To judge studies which have not been performed according to these guidelines, criteria were developed [3]. PAHs are characterized by low to very low solubility and therefore special attention is paid to the way the solutions for experiments have been prepared.

The laboratory toxicity data reviewed here are limited to the following PAHs: naphthalene, anthracene, phenanthrene, fluoranthene, benz[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and benzo[*ghi*]perylene. Data are summarized in Tables 6–8 and are presented as L(E)C50 for short-term tests with a duration of four days or less, and NOEC for long term tests with a duration greater than four days. For microorganisms and algae, however, NOEC may be derived from results of experiments lasting less than four days.

The LC50 is the Lethal Concentration for 50% of the organisms in the test (concentration at which 50% of the organisms tested die). LC50 values are often estimated applying a model to the data (e.g. [109]). The EC50 is the 50% Effect Concentration (concentration at which 50% effect occurs, e.g. a 50% reduction of growth). EC50 values are often estimated applying a log-logistic model (e.g. [106]). The NOEC is the No Observed Effect Concentration (highest concentration/dose in a series of test concentrations causing no significant adverse effect compared with the control). This concentration is often derived by comparing the effect at the tested concentrations statistically with the control (e.g. [105]).

One value per endpoint for a certain species is presented in the tables. If for one test species several toxicity data based on the same toxicological endpoint are available, these values are averaged by calculating the geometric mean. For all the PAHs considered, fewer NOEC values than L(E) C50 were found in the literature.

The toxicity data on freshwater species are presented in Table 6. For naphthalene, anthracene, phenanthrene and fluoranthene data were found for at least four taxonomic groups. For benz[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and benzo[*ghi*]perylene the number of data is much lower. The toxicity data on marine species are presented in Table 7. The number of data available for marine species is much lower than for freshwater species, and most data concern naphthalene. For benz[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and benzo[*ghi*]perylene, no toxicity data at all are available.

For terrestrial species even less toxicity data are available. Most data concern anthracene, phenanthrene and benzo[*a*]pyrene (Table 8). As was mentioned above, only those studies are included in which a negative effect compared to the control was observed. For some terrestrial isopod species, however, positive effects on reproduction have been reported [80, 317]; such effects were not included in the NOEC values summarized in Table 8.

14.4.2 Evidence for Specific Toxicity Mechanisms

The toxicity mechanisms reviewed in Sect. 14.3 of this chapter can be divided into (1) non-specific toxicity mechanisms (narcosis) and (2) more specific toxi-

Table 6. Toxicity of PAHs for freshwater organisms

PAH	taxonomic group	species	parameter	value (µg/l)	Reference
naphthalene	Chlorophyta	*Chlorella vulgaris*	EC50 growth	33000	[152]
	Mollusca	*Physa gyrina*	LC50	5000	[216]
	Insecta	*Chironomus attenuattus*	LC50	13000	[58]
		Chironomus tentans	EC50 immobility	2800	[216]
		Tanytarsus dissimilis	LC50	12600	[58]
		Somatochlora cingulata	LC50	1000–2500	[51]
	Crustacea	*Daphnia magna*	LC50	8799[a]	[52; 28; 75; 1]
		Daphnia magna	EC50 immobility	2200[a]	[216; 226]
		Daphnia pulex	LC50	3879[a]	[312; 96; 97; 98]
		Daphnia pulex	EC50 immobility	4700	[291]
	Pisces	*Pimephalis promelas*	NOEC hatchability	450	[65]
		Pimephalis promelas	NOEC mortality	1800	[65]
		Pimephalis promelas	NOEC growth	450	[65]
		Sarotherodon mossambicus	NOEC growth	2300	[56]
		Micropterus salmoides	LC50	680	[216]
		Oncorhynchus mykiss	LC50	120	[216]
		Pimephalis promelas	LC50	2693[a]	[95; 216; 65]
		Oreochromi mossambicus	LC50	7900	[55]
	Amphibia	*Xenopus laevis*	LC50	2100	[76]
anthracene	Chlorophyta	*Selenastrum capricornutum*	NOEC growth	3.6	[94]
		Selenastrum capricornutum	NOEC prim. production	2.5[a]	[94]
	Angiospermae	*Lemna gibba*	NOEC	300	[133]
	Insecta	*Aedis aegypti*	LC50	64	[251; 146]
	Crustacea	*Daphnia magna*	NOEC growth	2.4[a]	[86]
		Daphnia magna	NOEC reproduction	1.7[a]	[86; 94]
		Daphnia magna	LC50	27	[145; 1]
		Daphnia magna	EC50 immobility	80–110	[226]
	Pisces	*Lepomis macrochirus*	LC50	3.9	[207; 248]
		Lepomis spec.	LC50	18	[248]

Table 6 (continued)

PAH	taxonomic group	species	parameter	value (µg/l)	Reference
anthracene	Pisces	*Pimephalis promelas*	NOEC hatchability	8.8	[108]
		Pimephalis promelas	LC50	360	[146]
phenanthrene	Chlorophyta	*Anabaena flos-aqua*	NOEC	600	[289]
		Selenastrum capricornutum	EC50	900	[289]
		Nitzschia palea	EC50	900	[289]
	Angiospermae	*Lemna gibba*	NOEC growth	600	[133]
	Crustacea	*Daphnia magna*	NOEC reproduction	57	[127]
		Daphnia magna	NOEC mortality	56	[127]
		Daphnia magna	NOEC growth	32	[127]
		Daphnia magna	EC50 immobility	516	[216; 226]
		Daphnia magna	LC50	596	[28; 75; 1]
		Daphnia pulex	NOEC reproduction	110	[96]
		Daphnia pulex	NOEC growth	60	[96]
		Daphmia pulex	EC50 immobility	505	[291; 255]
		Daphmia pulex	LC50	338	[312; 98]
	Pisces	*Brachydanio rerio*	NOEC growth	42	[127]
		Brachydanio rerio	NOEC mortality	> 56	[127]
		Micropterus salmoides	LC50	250	[216]
		Oncorhynchus mykiss	LC50	30	[216]
fluoranthene	Cyanophyta	*Anabaena flos-aqua*	NOEC	50	[289]
	Chlorophyta	*Selenastrum capricornutum*	EC50	54000	[289]
	Angiospermae	*Lemna gibba*	NOEC growth	200	[268]
	Insecta	*Aedis aegypti*	LC50	12	[146]
		Chironomus tentans	EC50	32	[305]
	Crustacea	*Daphnia magna*	EC50	100	[305]
		Hyalella azteca	EC50	45	[305]
	Pisces	*Brachydanio rerio*	NOEC mortality	69	[127]
		Brachydanio rerio	NOEC growth	12	[127]
		Pimephalis promelas	LC50	200	[146]

Table 6 (continued)

PAH	taxonomic group	species	parameter	value (µg/l)	Reference
benzo(*a*)anthracene	Crustacea	*Daphnia pulex*	LC50	10	[312]
chrysene	Crustacea	*Daphnia magna*	NOEC-mortality	> 1.4	[124]
		Daphnia magna	NOEC-reproduction	> 1.4	[124]
benzo(*k*)fluoranthene	Pisces	*Brachydanio rerio*	NOEC-mortality	0.48	[126]
		Brachydanio rerio	NOEC-growth	0.36[a]	[126]
benzo(*a*)pyrene	Pisces	*Brachydanio rerio*	NOEC	6.3	[128]
	Chorophyta	*Scenedesmus capricornutum*	NOEC-growth	10	[44]
		Scenedesmus capricornutum	EC50-growth	5.7[a]	[277; 44]
	Crustacea	*Daphnia pulex*	LC50	5.0	[312]
benzo(*ghi*)perylene	Pisces	*Pimephalis promelas*	LC20	0.15	[249]

[a] geometric mean of results from different tests.

Table 7. Toxicity of PAHs for marine organisms

PAH	taxonomic group	species	parameter	value (µg/l)	Reference
naphthalene	Bacteriophyta	*Photobacterium phosphoreum*	EC50	2792[a]	[11]
	Rhodophyta	*Champia parvula*	NOEC	< 350	[310]
	Mollusca	*Callinectus sapidus*	LC50	2337[a]	[273]
	Crustacea	*Cancer magister*	NOEC larv. development	21	[37]
		Artemia salina	EC50-immobility	3200	[89]
		Calanus finmarchicus	LC50	1400	[81]
		Elaspomus pectenicrus	LC50	2700	[184]
		Eurytemora affinis	LC50	3800	[252]
		Hemigraphus nudus	LC50	1863[a]	[100]
		Neomysis americana	LC50	1051[a]	[290]

Table 7 (continued)

PAH	taxonomic group	species	parameter	value (µg/l)	Reference
naphthalene	Crustacea	*Artemia salina*	LC50	11000	[1]
		Palaemonetes pugio	LC50	2600	[7]
		Parhyale hawaiensis	LC50	10416[a]	[184]
		Penaeus aztecus	LC50	2500	[7]
	Annelida	*Neanthus arenaceodentata*	LC50	3800	[272]
	Pisces	*Oncorhynchus gorbusscha*	NOEC-growth	259[a]	[221]
		Oncorhynchus kisutch	NOEC-growth	370	[220]
		Fundulus heteroclitus	LC50	5300	[69]
		Gadus morhua	LC50	750	[81]
		Metapenaeus monocerus	LC50	5087[a]	[66]
		Cyprinodon variegatus	LC50	2400	[7]
		Oncorhynchus gorbuscha	LC50	900	[309]
anthracene	Bacteriophyta	*Photobacterium phosphoreum*	EC50	11	[11]
	Crustacea	*Artemia salina*	LC50	32[a]	[1; 146]
phenanthrene	Bacteriophyta	*Photobacterium phosphoreum*	EC50	255[a]	[11]
	Annelida	*Nanthes arenaceodentata*	LC50	600	[272]
	Crustacea	*Rhithropanopeus harrissi*	NOEC-mortality	150	[176]
		Artemia salina	LC50	680	[1]
		Artemia salina	EC50-immobility	520	[89]
fluoranthene	Bacteriophyta	*Photobacterium phosphoreum*	EC50	470	[11]
	Annelida	*Neanthes arenaceodentata*	LC50	300	[272]
	Crustacea	*Artemia salina*	LC50	40	[146]

[a] geometric mean of results from different tests.

Table 8. Toxicity of PAHs for terrestrial species

PAH	taxonomic group	species	parameter	value (mg/kg)	Reference
naphthalene	Macrophyta	*Latuca sativa*	EC50-shoot growth	± 100	[134]
anthracene	Macrophyta	*Avena sativa*	LC50	530	[217]
		Avena sativa	EC50-growth	30	[217]
		Cucumis sativus	EC50-growth	720	[217]
		Cucumis sativus	LC50	> 1000	[217]
		Glycine max	EC50-growth	> 1000	[217]
		Glycine max	LC50	> 1000	[217]
		Brassica ericifolia	EC50-growth	> 1000	[217]
		Brassica ericifolia	LC50	> 1000	[217]
		Casuarina distyla	EC50-growth	> 1000	[217]
		Casuarina distyla	LC50	> 1000	[217]
		Eucalyptus eximia	EC50-growth	> 1000	[217]
		Eucalyptus eximia	LC50	> 1000	[217]
phenanthrene	Annelida	*Eisenia fetida*	EC50	240	[30]
	Insecta	*Folsomia candida*	NOEC-reproduction	75	[30]
		Folsomia candida	LC50	150	[30]
benzo(*a*)anthracene	Crustacea	*Oniscus asellus*	NOEC-weight	7.5	[317]
	chrysene Annelida	*Eisenia fetida*	LC50	> 1000	[30]
benzo(*a*)pyrene	Annelida	*Enchytraeus crypticus*	NOEC-reproduction	3.4	[2]
		Eisenia fetida	NOEC-survival	1.0	[2]
	Crustacea	*Oniscus asellus*	NOEC-growth	32	[316]
		Porcellio scaber	NOEC-growth	28[a]	[335; 316]

[a] geometric mean of results from different tests.

city mechanisms involving photochemical or biochemical activation and subsequent adduct formation, or disturbance of hormonal regulation. According to the chemical classification scheme of Verhaar et al. [346] PAHs act by aspecific (narcosis) toxicity. The toxicity data compiled in Tables 6 and 7 have been used to test this hypothesis of aspecific toxicity of PAHs.

If narcosis-like mode of action is responsible for the toxicity of PAHs observed in experimental studies, the toxicity of PAHs should increase with increasing log K_{ow} (see Sect. 14.3.1, [346]). The aquatic toxicity for the different PAHs, based on the L(E)C50 values for freshwater and marine organisms (Tables 6 and 7) is compared visually in Figs. 5 and 6 for freshwater and marine species respectively. The PAHs are arranged in order of increasing log K_{ow}. Log K_{ow}values have been derived from the MEDCHEM database [214]. Although

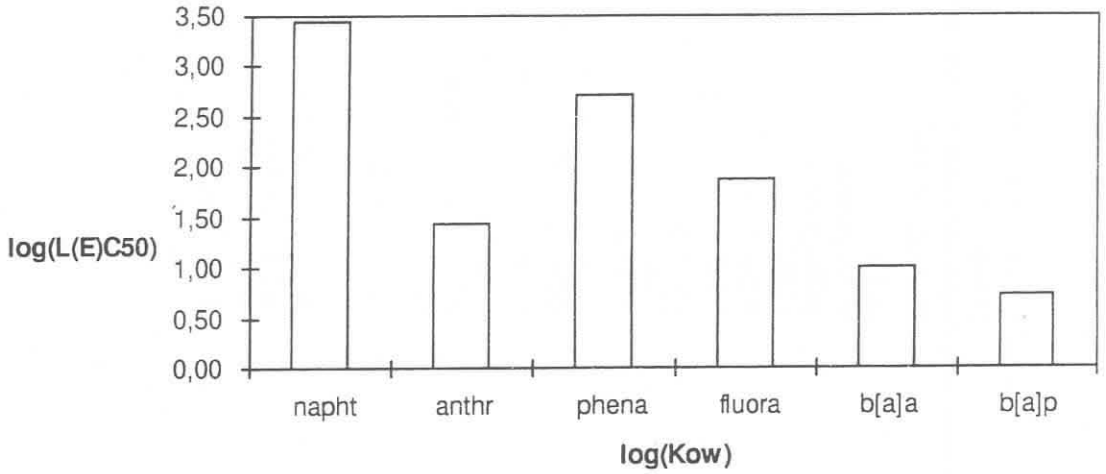

Fig. 5. Comparison of L(E)C50 values for different freshwater species and endpoints for the different PAHs considered (napht = naphthalene, anthr = anthracene, phena = phenanthrene, fluora = fluoranthene, b[*a*]a = benzo[*a*]anthracene, ben[*a*]p = benzo[*a*]pyrene)

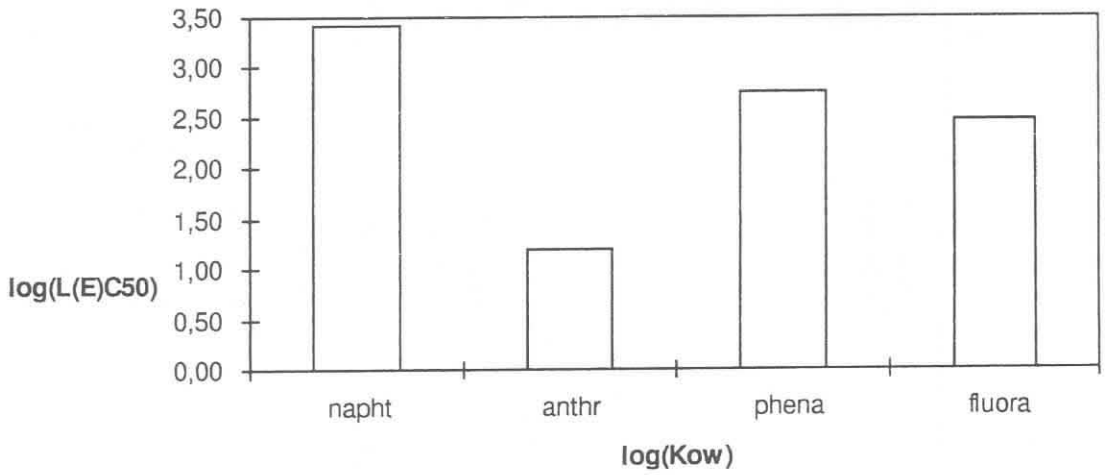

Fig. 6. Comparison of L(E)C50 values for different marine species and endpoints for the different PAHs considered (napht = naphthalene, anthr = anthracene, phena = phenanthrene, fluora = fluoranthene)

only few data are available, it is clear that the logL(E)C50 of PAHs for both marine and freshwater species generally decreases with an increasing log K_{ow}. Anthracene is an exception, and is more toxic than would be expected on the basis of its K_{ow}. This implies either that the toxicity of anthracene is caused by a more specific toxicity mechanism and not by narcosis, or the narcotic potency of anthracene is underestimated using K_{ow}.

If PAHs act by non-specific (narcosis) toxicity, the toxicity of PAHs can be accurately predicted on the basis of QSARs developed for narcotic chemicals. The toxicity observed in laboratory studies was compared with the toxicity predicted on the basis of the QSAR estimates for the toxicity of narcotic chemicals [148, 149]. Although QSARs are available for 19 different species [333], the comparison (Table 9) is limited to 7 PAHs and 4 species, since experimental data are scarce. In each case the QSAR-NOEC and experimental NOEC compared are based on the same effect parameter, i.e. growth. To express the differences between the experimental values and QSARs, the quotient is given of the predicted QSAR-NOEC and the experimentally determined NOEC.

For naphthalene, phenanthrene, fluoranthene, chrysene and benzo[*a*]pyrene, QSAR-NOECs and experimental NOECs are fairly comparable and this implies that non-specific (narcosis) toxicity is the mechanism responsible for the toxicity observed in these experimental studies. The quotient for these substances varied from 1.2 to 2.5 and was less than 3.6. For anthracene and benzo[*k*]fluoranthene, however, the experimental NOECs clearly deviate from the QSAR predicted values. This means that the toxicity of anthracene and benzo[*k*]fluoranthene cannot be explained on the basis of nonpolar narcosis and that consequently the toxicity of these compounds is caused by a more specific mechanism. Both compounds have been shown to be phototoxic (Table 5, [11, 215])

The data set reviewed here contains a considerable bias towards values obtained in short term toxicity studies. Under such conditions, nonpolar narcosis is the most likely mechanism of toxicity to be observed. Effects due to other

Table 9. Comparison of predicted QSAR-NOECs according to Van Leeuwen et al. [333] with experimental NOECs for different PAHs and species. After Kalf et al. [149]

PAH	species	QSAR-NOEC (µg/l)	exp.-NOEC (µg/l)	quotient (QSAR/exp.)
naphthalene	*Pimephalis promelas*	770	450	1.7
anthracene	*Selenastrum capricornutum*	120	3.6	33
	Daphnia magna	84	1.7	50
phenanthrene	*Daphnia magna*	82	57	1.4
	Brachydanio rerio	105	42	2.5
fluoranthene	*Brachydanio rerio*	29	12	2.4
chrysene	*Daphnia magna*	5 ≥	1.4	≤ 3.6
benzo(*k*)fluoranthene	*Brachydanio rerio*	6.8	0.36	19
benzo(*a*)pyrene	*Brachydanio rerio*	7.2	6.3	1.1

toxicity mechanisms, such as biochemical activation and subsequent adduct formation, and disturbance of hormonal regulation, will be missed as it will take a longer exposure period for effects to be observed at the individual level. Observations of phototoxicity are unlikely as the light sources used in the experiments reviewed here are generally not representative of outdoor conditions. Under natural conditions, with long term exposure of organisms to PAHs, more specific and therefore higher toxicity may be expected, than has generally been documented in the literature. The ecotoxicological consequences of PAH exposure can therefore not be properly assessed until more chronic data become available. In addition, a theoretical framework needs to be developed to deal with such long term chronic effects as carcinogenesis and mutagenesis, as the consequences of these on the population and ecosystem level are still poorly understood. Histopathological evidence for such specific toxic effects of PAHs are reviewed by De Maagd and Vethaak [Chap. 15].

The experimental evidence for disturbance of hormonal regulation is quite limited. The studies by Faber and Heijmans [80] and Van Brummelen et al. [317] suggest that a stimulation of reproduction of terrestrial isopods exposed to PAHs may occur and this may be due to disturbance of hormonal regulation. Both studies have not been included in the tables as the data set compiled was restricted to studies in which survival, growth and/or reproduction is decreased compared to the control.

14.4.3 Sublethal Sensitivity Index

In the previous section, it was pointed out that the data set reviewed here contains a considerable bias towards results from short term toxicity studies. Certain types of effects of PAHs are not taken into account in this data set, whereas these will occur in the environment as a result of prolonged exposure to relatively low concentrations.

To give some idea of the order of magnitude involved, the data set was investigated for species where both acute and semi-chronic data are available. The Sublethal Sensitivity Index (SSI) can be defined as the quotient of the acute lethality or LC50 value, and a chronic sublethal parameter, e.g. NOEC growth or reproduction [53]. According to the hypothesis of Daniels and Allan [4, 57] population growth shows little impairment up to the concentration at which lethality occurs, provided that sublethal effects on reproduction and lethal effects occur at concentrations which are close to each other, i.e. when SSI is low. Population impairment may occur at concentrations well below lethality when the SSI is higher.

From the data sets in Tables 6 and 7 it was possible to calculate SSIs for reproduction of *Pimephalis promelas* exposed to naphthalene (7.8), *Daphnia magna* exposed to anthracene (16) and phenanthrene (18), and *D. pulex* exposed to phenanthrene (3.1): the mean SSI was 10. These SSIs have been calculated by combining chronic and acute studies from different authors, which is not entirely satisfactory since the additional variance among laboratories can be partly responsible for the observed difference between the chronic and the

acute effect concentration. Based on the limited, biased data set, the standard factor of 10 often used in risk assessment for extrapolation from acute to chronic effect concentrations [332] appears to reflect approximately the average difference between chronic and acute studies. Such extrapolation factors are often called ‚safety factors', although in this case the factor 10 does not reflect a worst case scenario. Again, the data set does not include carcinogenic and mutagenic effects which have been shown to emerge at much lower concentrations.

14.4.4 Toxicity Towards Marine and Freshwater Species

For a number of compounds, salinity has an important influence on toxicity. To investigate whether this is also the case for PAHs, marine and freshwater toxicity data can be compared. As the data sets for most of the PAHs considered are rather small, it is not possible to make such a comparison on the basis of NOECs. Only for naphthalene are enough experimental L(E)C50s available to make a comparison of the sensitivity distributions.

Figure 7 presents the comparison of freshwater and marine species. For each species, one value is included in this figure. If more than one value is available for a species, the lowest value is used. A comparison of the data on the basis of a t-test showed no significant difference in sensitivities for naphthalene between freshwater and marine species ($p>0.05$). It is not expected that the sensitivities of freshwater and marine species to other PAHs will lead to another conclusion.

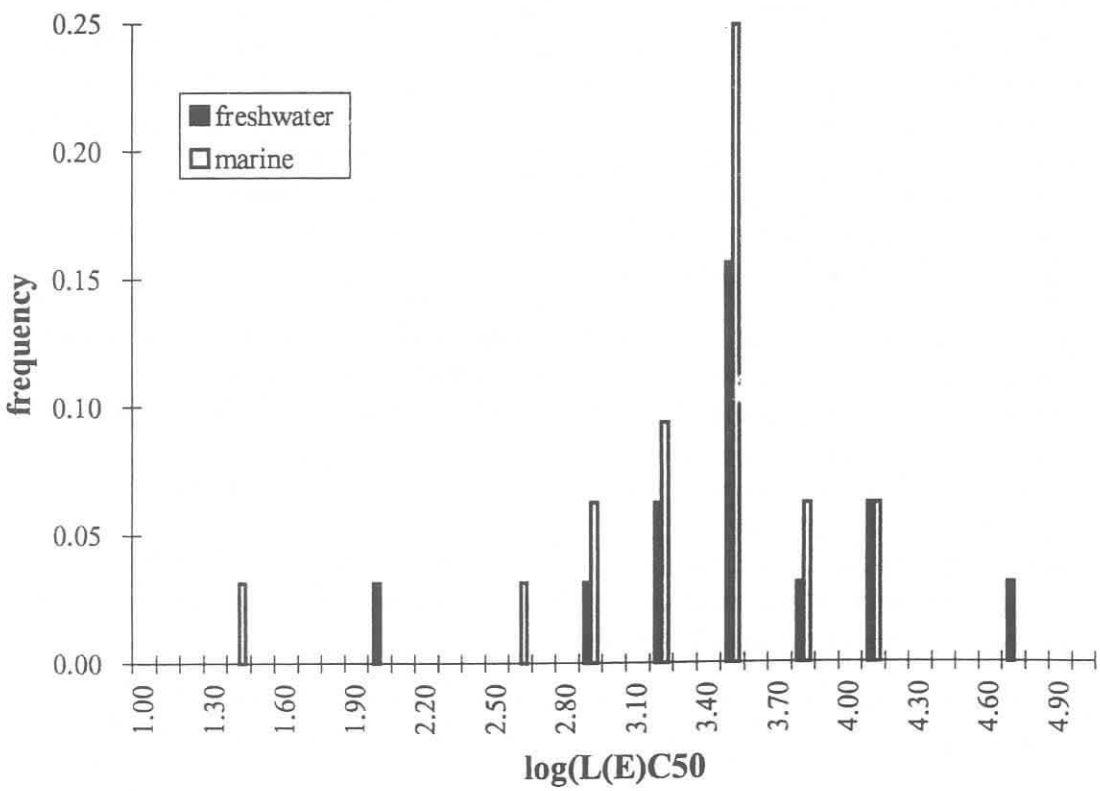

Fig. 7. Comparison of the sensitivities of freshwater and marine species for naphthalene

14.4.5
Environmental Quality Guidelines for PAHs and Mixture Toxicity

As indicated in the introduction to this section, ecotoxicity data are often used in risk assessment to derive a concentration below which, with a certain probability, no effects on ecosystems are expected. These concentrations are indicators of potential risk and may be used as guidelines in environmental policy and are often referred to as environmental quality guidelines, environmental quality objectives or environmental quality criteria. For the sake of convenience it will be referred to as quality guidelines in this chapter.

As a first step, concentrations of PAHs encountered in the environment are usually compared with these quality guidelines or with the underlying toxicity data. A comparison with a concentration in the field will tell us if a potential risk can be expected. When the concentrations are so high that a significant potential risk is present for biota, one should focus on the actual risk. The distinction between potential and actual risk is important: the former is based on laboratory toxicity data using matrices freshly spiked with PAHs which are therefore readily available for uptake by biota. The latter takes into account reductions in bioavailability (see Sect. 14.2) which may occur due, for example, to aging, or to incorporation of PAHs in pitch or coke globules [253].

Environmental guidelines for PAHs have been developed in order to assess the risk of concentrations of PAHs encountered in the field [148, 314]. Most quality guidelines are based on effects on survival, reproduction and growth encountered in the laboratory, while carcinogenic and mutagenic effects on ecosystems are ignored. The number of toxicity data available is small and consists mostly of short-term toxicity studies. Significant safety factors are usually applied for the derivation of these quality guidelines.

For the aquatic environment enough data for the development of quality criteria are available. For sediment and soil, the development of environmental quality criteria is severely hampered by the limited data on effects of PAHs. In this case the quality criteria for sediment and soil may be derived from aquatic quality criteria, using the equilibrium partitioning method [71].

The use of the equilibrium partitioning method for deriving sediment quality criteria is however a subject of discussion [9, 135]. The use of this method is based on the following assumptions:

1. that bioavailability, toxicity and bioaccummulation of nonionic organic chemicals are closely related to concentrations in the pore water;
2. that pore water concentrations are inversely proportional to the total particulate matter content in sediments;
3. that an equilibrium exists between the concentration of the compound in pore water and sediment;
4. that aquatic organisms have a similar sensitivity to PAHs as sediment dwelling organisms and can therefore be used to derive sediment quality criteria with an appropriate level of protection.

For soil, the use of the equilibrium partitioning method is also debatable. In some areas within the soil the amount of water is very small and may vary with

weather conditions, giving rise to non-equilibrium conditions. Another confounding factor is the role of direct uptake from food which may be important for compounds with a log K_{ow} higher than 5, as was shown for earthworms by Belfroid et al. [20, 22]. The limited number of terrestrial toxicity data is not likely to change significantly in the near future despite the fact that soils and sediments are major sinks for PAHs in the environment (Mackay, Chap. 8; Simoneit, Chap. 5). The use of the equilibrium partitioning methodology serves as a pragmatic but imperfect solution to this lack of data.

Another approximate way of deriving sediment guidelines has been elaborated by Long et al. [195] using the "Biological Effects Databases for Sediments" or BEDS. In these BEDS, effect data from equilibrium partitioning studies, laboratory spiked-sediment bioassays and field studies of sediment toxicity and benthic community composition are included. On the basis of the BEDS data set, guidelines are derived by quantifying the incidence of adverse effects within concentration ranges. This method is being used as a basis for developing National Sediment Quality Guidelines for Canada and Florida.

Most quality guidelines for PAHs have been developed for individual compounds, whereas in the field mixtures are almost invariably found. For compounds that have a similar structure it is often assumed that they have a similar toxicity mechanism, in which case the Toxic Unit Model can be used to predict the toxicity of mixtures [107, 120, 194, 236, 307]. Quality criteria for PAHs using these models have been proposed. A major drawback of these models is the assumption that a similar mode of action operates for all PAHs. As was indicated in previous sections of this chapter, this assumption is not valid: PAHs act by different modes of action. This means that the proposed models are not valid and should not be used. No suitable alternative is yet available to derive effect concentrations for mixtures of PAHs.

14.5 Determining the Actual Risk and Biological Effects of PAH Contamination

In both the aquatic and the terrestrial environments, man is confronted with areas having elevated concentrations of PAHs in the soil and in sediments. A comparison of these concentrations with toxicity data may reveal a potential risk for biota. The issue of concern is whether life and ecosystem processes are indeed adversely affected by these contaminants. To answer this question, a stepwise approach is advocated here using a procedure that integrates chemical and biological effects.

14.5.1 Determining the Actual Risk

The actual risk is largely determined by the bioavailability of the PAH contamination. As was indicated in Sect.14.2, the in-situ tissue residues of PAHs can be used as a measure of the bioavailability and mobility of contaminants. We have demonstrated, however, that species specific information is needed for interpretation of residue levels in terms of bioavailability. When interested in the

availability to organisms in general, it is advisable to measure PAH residue levels only in those species that have a low metabolic capacity: invertebrates, and particularly mollusks are more suitable than fish. As the fraction of contaminants available varies between species, species should be selected with different uptake pathways for which extensive literature on environmental residue levels and uptake/clearance kinetics is available.

In the marine environment, molluscs such as *Mytilus edulis* and *Crassostrea virginica* are very suitable organisms for this purpose as they are widely used in monitoring and research programs [25, 83, 175, 238, 303, 349]. For fresh water systems, much less data are available; the crustaceans *Diporeia* [166], *Asellus aquaticus* [329] and daphnids [75, 329] and the mollusk *Dreissena polymorpha* [34, 60] are possible candidates. In the terrestrial environment even less information is available on tissue residues of PAHs. Earthworm species, such as *Lumbricus rubellus*, can be used and their residues compared the data of Van Brummelen et al. [320]. Valuable information can also be found in work by Faber and Heijmans [80].

The biological variation in residue levels among animals collected from the field can be reduced by active biological monitoring: translocating a homogeneous group of animals from a reference population or a laboratory stock to the research sites (e.g. [60]). Alternatively, sediment or soil samples can be transferred to the laboratory where the accumulation of PAHs is determined after exposure of laboratory animals for a restricted period under controlled conditions (bioaccumulation assay).

The bioavailability of PAHs to animal species with a higher capacity for metabolism can be assessed by the analysis of PAH metabolites. This has been done successfully in bile from both marine and freshwater fish [13–15, 117, 159–161, 189, 324, 325], in hemolymph of crabs [253] and methods are under development for the measurement of PAH metabolites in tissue from terrestrial invertebrates [304]. The literature on applications of these techniques to field samples is very limited and as a consequence the results of this type of research are difficult to interpret. PAH metabolites however do not reflect only bioavailability, as they are closely linked to one of the mechanism of PAH toxicity: biochemical activation and subsequent adduct formation. A correlation of PAH metabolite levels and hepatic tumours has been observed by Krahn et al. [161].

A comparison of residue levels of PAHs or their metabolites from different locations will show which of the locations has the highest degree of bioavailable contamination. If sufficient literature is available, it is possible to assess whether the concentrations fall into the lower or the higher range of residue levels reported for a particular species. Residue levels cannot yet reveal the existence of adverse effects on an organism that result from the contamination. This may change when more data become available on internal effect concentrations [204, 206].

14.5.2 Biological Effects

Biological effects of a mixture of contaminants at a field site can be assessed using bioassays. For specific application to PAHs, bioassays have disadvantages

since they generally do not have diagnostic value, and are expensive when deployed to reveal long term chronic effects. The use of biochemical markers is generally cheaper and offers some diagnostic value. A disadvantage of biochemical markers (e.g. MFO activity) is that these detect changes at the biochemical level, which may not be indicative of adverse health effects per se, as these effects may be within the tolerance limits of organisms [257, 260]. On the one hand this allows the use of these techniques as early warning indicators [259], e.g. as a tool for estimating the geographical magnitude of any potential impacts [256, 257]. On the other hand the question is raised what the ecological meaning of a biochemical marker response is: at what stage do biochemical effects change from harmless into harmful, causing adverse effects at the individual or population level [257, 259, 328]? In our opinion the result of biomarker research is often difficult to interpret in ecological terms. A way to deal with this problem is to apply the precautionary principle and classify biochemical changes above specific benchmark values as undesirable adverse effects. The combination of bioassays with biomarkers, although expensive, forms a desirable combination of ecologically meaningful information with diagnostic value.

Within the framework of the Oslo and Paris Conventions for the Prevention of Marine Pollution (OSPAR) and in collaboration with the International Council for Exploration of the Sea (ICES), a PAH-specific monitoring program of biological effects has been designed. In a recent OSPAR/ICES workshop on biological effects monitoring techniques held in Aberdeen [10], a suite of three biomarker techniques was proposed to describe the impact of PAH compounds on biota at the biochemical level. The biomarker techniques selected by the workshop include cytochrome P450-1A [295], bulky aromatic-DNA adducts and PAH metabolites in bile. A suite of techniques is recommended since these three techniques individually do not register effects exclusively due to PAHs, e.g. cytochrome P450-1A activity may be induced not only by anthropogenic PAHs but probably also by PAHs produced from natural steroid and terpenoid precursors (Simoneit, Chap. 5 and Neilson and Hynning, Chap. 6) and PCBs and dioxins [35].

This suite of biomarkers can be used to measure exposure and biochemical effects. According to the conclusion of the workshop, PAH metabolites in bile are mainly a marker of exposure whereas the induction of cytochrome P450-1A also indicates effect, since it reveals a biochemical change within the organism which often precedes the onset of more serious cellular and physiological changes including hepatic damage, reproductive toxicity and immunotoxicity. The occurrence of DNA adducts is scored by the workshop as a deleterious effect since their formation is believed to be important in the initiation of carcinogenesis (see also [338; Cavalieri and Rogan, Chap. 11; de Maagd and Vethaak, Chap. 15). When the selected suite of biomarkers demonstrates a PAH-related biochemical response, subsequent histopathological examination of liver samples is recommended by the workshop. This technique can give additional information on deleterious effects of PAH exposure at the individual level; see de Maagd and Vethaak, Chap. 15, for further details.

The cytochrome P450 assay has recently been reviewed by Buchelli and Fent [35] and is probably the biomarker which has been used most frequently in

field trials. The performance of this biomarker is often judged by correlating the observed responses with external concentrations in sediment or water. Only in some of the field studies was a clear relationship found between cytochrome P450 activity and environmental concentrations, e.g. Stegeman et al. [299] found a positive correlation of EROD activity in Baltic flounder (*Platichthys flesus*) with PCBs and PAHs in mussels from the same site. Goksøyr et al. [102] found a correlation of a pollution gradient with increased EROD activity and cytochrome P450-1A content. A positive correlation was also found for EROD activity in comber (*Serranus cabrilla*) and sediment PAH content [228]. The limited number of field studies demonstrating such clear correlations is difficult to interpret. It may mean that this biomarker does not perform well, as has been demonstrated under certain conditions [35, 79, 288, 324]. Alternatively it may mean that external concentrations are not a good measure of actual exposure or risk, which has been argued throughout this chapter and was one of the main reasons for developing such biomarkers in the first place.

An assessment of the biological effects of a PAH contamination will be based on the weight of evidence from the suite of biomarkers and chemical concentrations in sediment, water and biota. This approach requires an integrated chemical and biological effects research program, and will profit from field experience at other contaminated sites.

The development of a PAH specific suite of biomarkers is an important step forward in the assessment of the biological effects of PAH contaminated field sites. The success of such an approach hinges on field experience and on the development of an interpretational framework defining at what stage biochemical effects change from harmless to harmful.

14.6 Conclusions

1. PAHs should not be regarded as a homogeneous group of chemicals. The environmental chemistry and toxicology of PAHs is highly complex and does not depend on simple physico-chemical characteristics. The key to an understanding of the environmental risk of PAHs is knowledge of the fraction available to the organism and the toxicity mechanisms involved, as these ultimately determine the magnitude and nature of biological effects.
2. The best way to assess PAH bioavailability is to determine the uptake rate constant via a kinetic study, although bioavailability is usually assessed on the basis of tissue residues. It is obvious that such an assessment can only be made if sufficient knowledge is available of kinetics and mechanisms of uptake, biotransformation and elimination.
3. Data from the scientific literature demonstrate that PAHs do not have one type of toxic action but that different toxicity mechanisms play a role, depending on the compound, the type of exposure (acute or chronic), the organism and the environmental conditions involved. Many PAHs can act through a more specific toxicity mechanism than nonpolar narcosis. Exceptions to this may be PAHs such as naphthalene, phenanthrene and fluorene, which are non-phototoxic, are not considered to be mutagens and do

not have a structure similar to hormones. PAHs with a higher molecular weight generally have longer residence times and have a more specific toxicological nature which may result in adverse effects during chronic exposure.

4. An overview of studies in which adverse effects of PAHs on reproduction, growth or survival are documented, reveals a considerable bias for short term toxicity studies. Under such conditions nonpolar narcosis is the most likely mechanism of toxicity to be observed. Effects due to other toxicity mechanisms, such as biochemical activation and disturbance of hormonal regulation, will be missed, as it will take a longer exposure period for effects to be observed at the individual level. Observations of phototoxicity are unlikely as the light sources used in most experiments are not representative of outdoor conditions. Under natural conditions, with long term exposure of organisms to PAHs, more specific and therefore higher toxicity can be expected than has generally been documented in the literature.
5. The ecotoxicological consequences of PAH exposure can therefore not be properly assessed until more chronic data become available. In addition, a theoretical framework needs to be developed to deal with such long term chronic effects as carcinogenesis and mutagenesis, as the consequences of these on the population and ecosystem level are still poorly understood.
6. The potential risk for biota of PAH contamination is usually assessed by comparing field concentrations with toxicity data from laboratory experiments.
7. The actual risk can be assessed by taking reductions in bioavailability due to, for example, aging into account. The in-situ tissue residues of PAHs or their metabolites can be used as a measure of the bioavailability and mobility of contaminants, provided species specific information is taken into account.
8. Biological effects of a mixture of contaminants at a field site can be assessed using biochemical markers which can detect changes at the biochemical level, but these changes may not be indicative of adverse health effects per se, as these effects may be within the tolerance limits of organisms. The development of a PAH specific suite of biomarkers is an important step forward in the assessment of the biological effects of PAH contaminated field sites. The success of such an approach hinges on field experience and on the development of an interpretational framework defining at what stage biochemical effects change from harmless to harmful.

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References

1. Abernethy S, Bobra AM, Shiu WY, Wells PG, Mackay D (1986) Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to two planktonic crustaceans: the key role of organism-water partitioning Aquat Toxicol 8:163
2. Achazi RK, Chroszcz G, Düker Chr, Henneken M, Rothe B, Schaub K, Steudel I (1995) The effect of fluoranthene (Fla), benzo[*a*]pyrene (BaP) and Cadmium (Cd) upon survival

rate and life cycle parameter of two terrestrial annelids in laboratory test systems. Newsletter on Enchytraeidae 4:7–14
3. ACT (1994) QA document for deriving environmental quality objectives (INS and I-values). Center for Substances and Risk Assessment, National Institute of Public Health and the Environment, Bilthoven, Netherlands. Rep ACT/KD/003 (In Dutch)
4. Allan JD, Daniels RE (1982) Life table evaluation of chronic exposure of *Eurytomora affinis* (Copepoda) to kepone. Mar Biol 66:179–184
5. Allred PM, Giesy JP (1984) Solar radiation induced toxicity of anthracene in *Daphnia pulex*. Environ Toxicol Chem 4:219–226
6. Anderson J, Birge W, Gentile J, Lake J, Rodgers J, Swartz R (1987) Biological effects, bioaccumulation and ecotoxicology of sediment-associated chemicals. In: Dickson KL, Maki AW, Brungs WA (eds.) Fate and effects of sediment-bound chemicals. Pergamon, New York, pp. 267–297
7. Anderson JW, Neff JM, Cox BA, Tatem HE, Hightower GM (1974) The effects of oil on estuarine animals: toxicity, uptake and depuration, respiration. Pollution and Physiology of Marine Organisms pp 285
8. Anderson RS (1979) Insectan aryl hydrocarbon hydroxylase generates benzo[*a*]pyrene metabolites that bind to protein and DNA. Comp Biochem Physiol 63 C:17–20
9. Ankley GT, Berry WJ, DiToro DM, Hansen DJ, Hoke RA, Mount DR, Reiley MC, Swartz RC, Zarba CS (1996) Use of equilibrium partitioning to establish sediment quality criteria for nonionic chemicals: a reply to Ianuzze et al. Environ Toxicol Chem 15: 1019–1024
10. Anonymous (1995) Report of the workshop on biological effects monitoring techniques, Aberdeen, Scotland, 2–6 October 1995. OSPAR registration number: SIME 96/3/1
11. Arfsten DP, Davenport R, Schaeffer DJ (1994) UV-A coexposure enhances the toxicity of aromatic hydrocarbons, munitions, and metals to *Photobacterium phosphoreum*. Biomed Environ Sci 7:101–108
12. Arfsten DP, Schaeffer DJ, Mulveny DC (1996) The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants. Ecotoxicol Environ Safety 33:1–24
13. Ariese F (1993) Shpol'skii spectroscopy and synchronous fluorescence spectroscopy – biomonitoring of polycyclic aromatic hydrocarbons and their metabolites. Thesis Vrije Universiteit, Amsterdam
14. Ariese F, Kok SJ, Verkaik M, Gooijer C, Velthorst NH, Hofstraat JW (1995) Synchronous fluorescence spectrometry of fish bile: a rapid screening method for the biomonitoring of polycyclic aromatic hydrocarbons in the aquatic environment. Aquat Toxicol 26: 273–286
15. Ariese F, Kok SJ, Verkaik M, Hoornweg GP, Gooijer C, Velthorst NH, Hofstraat JW (1993) Chemical derivatization and Shpol'skii spectrofluorometric determination of benzo[*a*]-pyrene metabolites in fish bile. Anal Chem 65:1100–1106
16. Barron MG, Stehly GR, Hayton WL (1990) Pharmocokinetic modeling in aquatic animals. I Models and concepts. Aquat Toxicol 18:61–68
17. Bast A, Haenen GRMM, Doelman CJA (1991) Oxidants and antioxidants: state of the art. Am J Med 91 (S3 C):2S–13 S
18. Bastian MV, Toetz DW (1982) Effect of eight polynuclear hydrocarbons on growth of *Anabaena flos-aquae*. Bull Environ Contam Toxicol 29:531
19. Baumann PC (1989) PAH metabolites and neoplasia in feral fish populations. In: Varanasi U (ed) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, Florida, pp 269–290
20. Belfroid A, Meiling J, Sijm D, Hermens J, Seinen W, Van Gestel K (1994) Uptake of hydrophobic halogenated hydrocarbons from food by earthworms (*Eisenia andrei*). Arch Environ Contam Toxicol 27:260–265
21. Belfroid A, Seinen W, Van den Berg M, Hermens J, Van Gestel K (1995) Uptake, bioavailability and elimination of hydrophobic compounds in earthworms (*Eisenia andrei*) in field contaminated soil. Environ Toxicol Chem 14:605–612

22. Belfroid A, Seinen W, Van Gestel CAM, Hermens JLM, Van Leeuwen KJ (1995) Modelling the accumulation of hydrophobic organic chemicals in earthworms. Environ Sci Pollut Res 2:5–15
23. Belfroid AC, Sijm DTHM, Van Gestel CAM (1996) Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. Environ Rev 4:276–299
24. Berdugo V, Harris RP, O'Hara SC (1977) The effects of petroleum hydrocarbons on the reproduction of an estuarine planktonic copepod in laboratory cultures. Marine Pollution Bulletin 8:138–143
25. Bergman A (1993) Concentrations of PAHs, PCBs and heavy metals in the blue mussel *Mytilus edulis*. Rijkswaterstaat, Tidal Waters Division, The Hague, Netherlands, Report 93.001
26. Bintein S, Devillers J, Karcher W (1993) Nonlinear dependence of fish bioconcentration on n-octanol/water partition coefficient. SAR and QSAR in Environ Res 3:22–39
27. Blair JM, Crossley DA Jr, Rider S (1989) Effects of naphthalene on microbial activity and nitrogen pools in soil-litter microcosms. Soil Biol Biochem 21:507–510
28. Bobra AM, Shiu WY, Mackay D (1983) A predictive correlation for the acute toxicity of hydrocarbons and chlorinated hydrocarbons to the water flea (*Daphnia magna*). Chemosphere 12:1121–1129
29. Bowling JW, Leversee G J, Landrum PF, Giesy JP (1983) Acute mortality of anthracene-contaminated fish exposed to sunlight. Aquat Toxicol 3:79–90
30. Bowmer CT, Roza P, Henzen L, Degeling C (1992) The development of chronic toxicological tests for PAH contaminated soils using the earthworm *Eisenia fetida* and the springtail *Folsomia candida*. TNO report IMW-R 92/387
31. Bowmer CT, Roza P, Henzen L, Degeling C (1993) The development of chronic toxicological tests for PAH contaminated soils using the earthworm *Eisenia fetida* and the springtail *Folsomia candida*. TNO-Rep IMW-R 92/387
32. Brattsten LB (1979) Ecological significance of mixed-function oxidations. Drug Metabol Rev 10:35–38
33. Broman D, Näf C, Ludbergh I, Zebühr Y (1990) An in-situ study of the distribution, biotransformation and flux of polycyclic aromatic hydrocarbons (PAHs) in an aquatic foodchain (seston – *Mytilus edulis* – *Somateria mollissima* L.) from the baltic: an ecotoxicological perspective. Environ Toxicol Chem 9:429–442
34. Bruner KA, Fisher SW, Landrum PF (1994) The role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling. 2 Zebra mussel contaminant accumulation from algae and suspended particles, and transfer to the benthic invertebrate, *Gammarus fasciatus*. J Great Lakes Res 20:735–750
35. Bucheli TD, Fent K (1995) Induction of cytochrome P450 as a biomarker for environmental contamination in aquatic ecosystems. Crit Rev Environ Sci Technol 25:201–268
36. Buhler DR, Williams DE (1989) Enzymes involved in metabolism of PAH by fishes and other aquatic animals: oxidative enzymes (or phase I enzymes). In: Varanasi U (ed) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, Florida, pp 151–184
37. Caldwell RS, Caldarone EM, Mallon MH (1977) Effects of a seawater-soluble fraction of cook inlet crude oil and its major aromatic components on larval stages of the Dungeness crab, *Cancer magister* Dana. In: Wolfe DA (ed). Fate and effects of petroleum hydrocarbons in marine organisms ecosystems. Pergamon, Oxford New York
38. Campbell PGC, Lesis AG, Chapman PM, Fletcher WK, Imber BE, Luoma SN, Stokes PM, Winfrey M (1988) Biologically available metals in sediments. Publ. No. 27694. National Research Council of Canada, Ottawa
39. Carman KR, Todaro MA (1996) Influence of polycyclic aromatic hydrocarbons on the meiobenthic-copepod community of a Louisiana salt marsh. J Exp Mar Biol Ecol 198: 37–54
40. Chessels M, Hawker DW, Connell DW (1992) Influence of solubility in lipid on bioconcentration of hydrophobic compounds. Ecotoxicol Environ Saf 23:260–273

41. Cid Montanes JF (1994) Analysis, environmental distribution and ecotoxicology of polycyclic aromatic hydrocarbons and modern biocides in the aquatic environment: an integrated study. Thesis, University of Barcelona Barcelona, Spain (in Spanish)
42. Cid Montanes JF, Deneer J, Van Hattum B (1995) Bioconcentration of chloropyrifos by freshwater isopods under time-varying exposure conditions in experimental ditches. Environ Pollut 88:137–146
43. Clements WH, Oris JT, Wissing TE (1994) Accumulation and food chain transfer of fluoranthene and benzo[*a*]pyrene in *Chironomus riparius* and *Lepomis macrochirus*. Arch Environ Contam Toxicol 26:261–266
44. Cody TE, Radike MJ, Warshawsky D (1984) The phototoxicity of benzo[*a*]pyrene in the green alga *Selenastrum capricornutum*. Environ Res 35:122–132
45. Coles JA, Farley SR, Pipe RK (1994) Effects of fluoranthene on the immunocompetence of the common marine mussel, *Mytilus edulis*. Aquat Toxicol 30:367–379
46. Connell DW (1990) Bioaccumulation of xenobiotic compounds. CRC-Press, Boca Raton, Florida
47. Connell DW, DW Hawker (1988) Use of polynomial expressions to describe the bioconcentration of hydrophobic chemicals by fish. Ecotoxicol Environ Saf 16:242–257
48. Connell, DW, Markwell RD (1990) Bioaccumulation in the soil to earthworm system. Chemosphere 20:91–100
49. Connor, MS (1984) Fish/sediment concentration ratios for organic compounds. Environ Sci Technol 18:31–35
50. Cornelissen G, Van Noort PCM, Govers HAJ (1997) Desorption kinetics of chlorobenzenes, PAHs and PCBs: sediment extraction with tenax and effects of contact time and solute hydrophobicity. Environ Tox Chem (in press)
51. Correa M, Coler R (1983) Enhanced oxygen uptake rates in dragonfly nymphs (*Somatochlora cingulata*) as an indication of stress from naphthalene Bull Environ Contam Toxicol 30:269–276
52. Crider JY, Wilhm J, Harmon HJ (1982) Effects of naphthalene on the hemoglobin concentration and oxygen uptake of *Daphnia magna* Bull Environ Contam Toxicol 28:52–57
53. Crommentuijn T, Doodeman CJAM, Doornekamp A, Van der Pol JJC, Rademaker MCJ, Van Gestel CAM (1994) Sublethal Sensitivity Index as an ecotoxicity parameter measuring energy allocation under toxicant stress. Application to cadmium in soil arthropods. Ecotoxicol Environ Saf 26:216–227
54. Curto Pons JM, Cid Montanes JF, Van Hattum B (1993) In situ bioaccumulation of polycyclic aromatic hydrocarbons by freshwater isopods in relation to partitioning between sediments, particulate matter and the water-phase. In: Garrigues P, Lammote M (eds): Polycyclic aromatic compounds synthesis, properties, measurements, occurence and biological effects. Polycycl Arom Compounds 3 – supplement. Gordon and Breach Sci Publ, Yverdon, Switzerland, pp 1031–1038
55. Dange AD (1986) Metabolic effects of naphthalene, toluene or phenol intoxication in the cichlid fish tilapia, *Oreochromis mosambicus*: Changes in aminotransferase activities. Environ Pollut (A) 42:311–323
56. Dange AD, Maserukar VB (1982) Naphthalene induced changes in carbohydrate metabolism in *Sarotherodon mosambicus* Peters (Pisces:Cichlidae). Hydrobiol 94:163–172
57. Daniels RE, Allan JD (1981) Life table evaluation of chronic exposure to a pesticide. Can J Fish Aquat Sci 38:485–494
58. Darville RG, Wilhm JL (1984) The effects of naphthalene on oxygen consumption and hemoglobin concentration in *Chironomus attaenuatus* and on oxygen consumption and life cycle of *Tanytarsus dissimilis*. Environ Toxicol Chem 3:135–141
59. De Guise S, Lagacé A, Béland P (1994) Tumors in St Lawrence beluga whales (*Delphinapterus leucas*). Vet Pathol 31:444–449
60. De Kock WC, Bowmer CT (1993) Bioaccumulation, biological effects and food chain transfer of contaminants in the zebra mussel (*Dreisena polymorpha*) In: Nalepa TF, Schloesser DW (eds) Zebra mussels – biology, impact and control. Lewis Publishers, London, pp 503–533

61. De Kock WC, Kramer KJM (1994) Active Biomonitoring (ABM) by translocation of bivalve molluscs. In: Kramer KJM (ed) Biomonitoring of coastal waters and estuaries. CRC Press, Boca Raton, Ann Arbor, London, Tokyo, pp 51–84
62. De Maagd GJ (1996) Polycyclic aromatic hydrocarbons: fate and effects in the aquatic environment. PhD Thesis University of Utrecht, Netherlands
63. De Maagd GJ, Sijm DTHM (1995) PAHs: chemical and toxicological properties in the aquatic environment. Research Institute for Toxicology, University of Utrecht, Netherlands (in Dutch)
64. De Voogt P, Van Hattum B, Leonards P, Klamer JC, Govers H (1991) Bioconcentration of polycyclic heteroaromatic hydrocarbons in the guppy (*Poecilia reticulata*). Aquat Toxicol 20:169–194
65. DeGraeve GM, Elder RG, Woods DC, Bergman HL (1982) Effects of naphthalene and benzene on fathead minnows and rainbow trout. Arch Environ Contam Toxicol 11:487–490
66. Deshmukh NV, Deshmukh VD, Maserukar VB (1985) Naphthalene toxicity to the speckled prawn, *Metapenaeus monocerus* (FAB): seasonal variations. Geobios 12:263–265
67. Dickman M, Brindle I, Benson M (1995) Evidence of teratogens in sediments of the Niagara River watershed as reflected by chironomid (Diptera: Chironomidae) deformities. J Gr Lakes Res 18:467–480
68. Dickson KL, Giesy JP, Parish R, Wolfe L (1994) Closing remarks of 13th Pellston Workshop, Pellston, Michigan, August 17–22 1992. In: Hamelink JL, Landrum PF, Bergman HL, Benson WH (eds) Bioavailability: physical, chemical and biological interactions. Lewis Publishers, Boca Raton pp 221–230
69. DiMichele L, Taylor MH (1978) Histopathological responses of *Fundulus heteroclitus* to naphthalene exposure. J Fish Res Board Can 35:1060–1066
70. Dinan L (1989) Ecdysteroid structure and hormonal activity. In: Koolman J (ed) Ecdysone, from chemistry to mode of action. Thieme Verlag, Stutgart New York
71. DiToro DM, Zabra CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR (1991) Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibium partitioning. Environ Toxicol Chem 10: 1541–1583
72. Dobroski CJ, Epifanio CE (1980) Accumulation of benzo[*a*]pyrene in larval bivalve via trophic transfer. Can J Fish Aquat Sci 37:2318–2322
73. Donkin P, Widdows J, Evand SV, Worral CM, Carr M (1989) Quantitative structure activity relationships of hydrophobic organic chemicals on rate of feeding by mussels (*Mytilus edulis*). Aquat Toxicol 14:277–294
74. Eadie BJ, Robbins JA, Landrum PF, Rice CP, Simmons MS, McCormick MJ, Eisenreich SJ, Bell GL, Pickett RL, Johansen K, Rossmann R, Hawley N, Voice T (1983) The cycling of toxic organics in the Great Lakes: a 3-year status report. NOAA Technical Memorandum, ERL GLERL-45. National Oceanic and Atmospheric Administration, Ann Arbor, Michigan, USA., 86:111–115
75. Eastmond DA, Booth GM, Lee ML (1984) Toxicity, accumulation and elimination of polycyclic aromatic sulfur heterocycles in *Daphnia magna*. Arch Environ Contam Toxicol 13:105
76. Edmisten GE, Bantle JA (1982) Use of *Xenopus laevis* larvae in 96 hour, flow through toxicity tests with naphthalene. Bull Environ Contam Toxicol 29:392–399
77. Eertman RHM, De Zwaan A (1994) Survival of the fittest: resistance of mussels to aerial exposure. In: Kramer KJM (ed) Biomonitoring of coastal waters and estuaries. CRC Press, Boca Raton, Ann Arbor, London, Tokyo, pp 269–284
78. Eertman RHM, Groenink CLFMG, Sandee B, Hummel H (1995) Response of the blue mussel *Mytilus edulis* L. following exposure to PAHs or contaminated sediment. Mar Environ Res 39:169–173
79. Eggens ML, Bergman A, Vethaak AD, Van der Weiden MEJ, Celander M, Boon JP (1995) Cytochrome P450–1A indices as biomarkers of contaminant exposure: results of a field study with plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) from the southern North Sea. Aquat Toxicol 32:211–225

80. Faber JH, Heijmans JSM (1996) Polycyclic aromatic hydrocarbons in soil detritivores. In: Van Straalen NM, Krivolutskii DA (eds) Bioindicator systems for soil pollution. Kluwer Academic Publishers, Dordrecht, pp 31–43
81. Falk-Petersen L, Saethre J, Lönning S (1982) Toxic effects of naphthalene and methylnaphthalenes on marine plankton organisms. Sarsia 67: 171–178
82. Farrington JW (1991) Biogeochemical processes governing exposure and uptake of organic pollutant compounds in aquatic organisms. Environ Health Perspect 90: 75–84
83. Farrington JW, Goldberg ED, Riseborough RW, Martin JH, Bowen VT (1983) U.S. "Mussel Watch" 1976–1978: an overview of the trace metals, DDE, PCB, hydrocarbon and artificial radio-nuclide data. Environ Sci Technol 17:490–496
84. Fernandez M, L'Haridon J (1992) Influence of lighting conditions on toxicity and genotoxicity of various PAH in the newt in vivo. Mutat Res 298:31–41
85. Ferraro SP, Lee H, Ozretich RJ, Specht DT (1990) Predicting bioaccumulation potential: a test of a fugacity-based model. Arch Environ Contam Toxicol 19:386–394
86. Foran JA, Holst LL, Giesy JP (1991) Effects of photoenhanced toxicity of anthracene on ecological and genetic fitness of *Daphnia magna*: a reappraisal. Environ Toxicol Chem 10:425–427
87. Forbes VE, Forbes TL, Holmer M (1996) Inducible metabolism of fluorathene by the oppurtunistic polychaete *Capitella* sp I. Mar Ecol Prog Ser 132:63–70
88. Foster GD, Baksi SM, Means JC (1987) Bioaccumulation of trace organic contaminants from sediment of baltic clams (*Macoma balthica*) and soft-shell clams (*Mya arenaria*). Environ Toxicol Chem 6:969–976
89. Foster GD, Tullis RE (1984) A quatitative-structure activity relationship between partition coefficients and the acute toxicity of naphthalene derivates in *Artemia salina* nauplii. Aquat Toxicol 5:245–254
90. Foster GD, Wright DA (1988) Unsubstituted polynuclear aromatic hydrocarbons in sediments, clams, and clam worms from Chesapeake Bay. Mar Pollut Bull 19:459–456
91. Frank AP, Landrum PF, Eadie BJ (1986) Polycyclic aromatic hydrocarbon rates of uptake, depuration, and biotransformation by Lake Michigan *Stylodrilus heringianus*. Chemosphere 15:317–330–367
92. Frez WA, Landrum PF (1986) Species-dependent uptake of PAH in Great Lakes invertebrates. In: Proceedings, 9th international symposium on polynuclear aromatic hydrocarbons. Batelle Press, Columbus, OH (USA), pp 291–304–323
93. Frumin GT, Chuiko GM, Pavlov DM, Menzykova OV (1992) New rapid method to evaluate the median effect concentrations of xenobiotics in hydrobionts. Bull Environ Contam Toxicol 49:361
94. Gala WR, Giesy JP (1992) Photo-induced toxicity of anthracene to the green algae, *Selenastrum capricornutum*. Arch Environ Contam Toxicol 23:316
95. Geiger DL, Northcott CE, Call DJ, Brooke LT (1985) Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). Centre for Lake Superior Environmental Studies, University of Wisconsin-Superior, vol 2
96. Geiger JG, Buikema AL (1982) Hydrocarbons depress growth and reproduction of *Daphnia pulex* (Cladocera). Can J Fish Aquat Sci 39:830–836
97. Geiger JG, Buikema AL, Cairns J Jr (1980) A tentative seven-day test for predicting effects of stress on populations of *Daphnia pulex*. In: Eaton, Parrish, Hendricks (eds) Aquatic Toxicology, ASTM STP 707, p13–26
98. Geiger JG, Buikema AL Jr (1981) Oxygen consumption and filtering rate of *Daphnia pulex* after exposure to water soluble fractions of naphthalene, Phenanthrene, No. 2 fuel oil, and coal-tar creosote. Bull Environ Contam Toxicol 27:783–789
99. Gerould S, Landrum PF, Giesy JP (1983) Anthracene bioconcentration and biotransformation in chironomids: effects of temperature and concentration. Environ Pollut 30: 175–188
100. Gharrett JA, Rice SD (1987) Influence of simulated tidal cycles on aromatic hydrocarbon uptake and elimination by the shore crab *Hemigrapsus nudus*. Mar Biol 95:365–370

101. Gobas FAPC, Zhang X (1992) Measuring bioconcentration factors and rate constants of chemicals in aquatic organisms under conditions of variable water concentrations and short exposure time. Chemosphere 25:1961–1971
102. Goksøyr A, Husøy A-M, Larsen HE, Klungsøyr J, Wilhelmsen S, Maage A, Brevik EM, Andersson T, Celander M, Pesonen M, Förlin L (1991) Environmental contaminants and biochemical responses in flatfish from the Hvaler archipelago in Norway. Arch Environ Contam Toxicol 21:486–496
103. Govers H, Ruepert C, Aiking H (1984) Quantitative structure-activity relationships for polycyclic aromatic hydrocarbons: correlation between molecular connectivity, physicochemical properties, bioconcentration and toxicity in *Daphnia pulex*. Chemosphere 13: 227–279
104. Grundy MM, Ratcliff NA, Moore MN (1996) Immune inhibition in marine mussels by polycyclic aromatic hydrocarbons. Mar Environ Res 42:187–190
105. Gulley DD, Boelter AM, Bergman HL (1989) Toxstat, release 3.0. Fish Physiology and Toxicology Laboratory, Wyoming
106. Haanstra L, Doelman P, Oude Voshaar JH (1985) The use of sigmoidal dose response curves in soil ecotoxicological research. Plant and Soil 84:293–297
107. Haas CN, Cidambi K, Kersten S, Wright K (1996) Quantitative description of mixture toxicity: effect of level of response on interactions. Environ Toxicol Chem 15:1429–1437
108. Hall AT, Oris JT (1991) Anthracene reduces reproductive potential and is maternally transferred during long-term exposure in fathead minnows. Aquat Toxicol 19: 249–264
109. Hamilton MA, Russo PC, Thurston RV (1977/1978) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–719 – Correction: Environ Sci Technol 12:417
110. Harkey GA, Landrum PF, Klaine SJ (1994) Preliminary studies on the effect of feeding during whole sediment bioassays using *Chironomus riparius* larvae. Chemosphere 28: 597–606
111. Harris RP, Berdugo V, O'Hara SCM, Corner EDS (1977) Accumulation of 14C-1-naphthalene by an oceanic and estuarine copepod during long-term exposure to low-level concentrations. Mar Biol 42:187–195
112. Hawker DW, Connel DW (1986) Bioconcentration of lipophilic compounds by some aquatic organisms. Ecotoxicol Environ Saf 11:184–197
113. Heitmuller PT, Hollister TA, Parrish PR (1981) Acute toxicity of 54 industrial chemicals to sheepshead minnows (*Cyprinodon variegatus*). Bull Environ Contam Toxicol 27:596
114. Hellou J, Upshall C, Ni IH, Payne JF, Huang YS (1991) Polycyclic aromatic hydrocarbons in harp seals (*Phoca groenlandica*) from the Northwest Atlantic. Arch Environ Contam Toxicol 21:135–140
115. Hellou J, Mackay D, Fowler B (1995) Bioconcentration of polycyclic aromatic compounds from sediments to muscle of finfish. Environ Sci Technol 29:2555–2560
116. Hellou J, Payne JF, Upshall C, Fancey LL, Hamilton C (1994) Bioaccumulation of aromatic hydrocarbons from sediments: a dose-response study with flounder (*Pseudopleuronectes americanus*). Arch Environ Contam Toxicol 27:477–485
117. Hellou J, Upshall C (1995) Monocyclic aromatic hydrocarbons in bile of flounder exposed to a petroleum oil. Internat J Environ Anal Chem 60:101–111
118. Hendriks AJ (1995) Modelling equilibrium concentrations of microcontaminants in organisms of the Rhine delta: can average field residues in the aquatic foodchain be predicted from laboratory accumulation? Aquat Toxicol 31:1–25
119. Hendriks J (1995) Concentrations of microcontaminants and response of organisms in laboratory experiments and Rhine delta field survey. PhD-thesis, University of Utrecht, Netherlands
120. Hensbergen PJ, Van Gestel CAM (1995) Combination toxicity in the terrestrial environment. Report of the Technical Commission for Soil Protection (in Dutch)
121. Hodgson E (1983) The significance of cytochrome P-450 in insects. Insect Biochem 13: 237–246

122. Holoubek I, Houskova L, Seda Z (1990) Project tocoen. The fate of selected organic pollutants in the environment-IV. Soil, earthworms and vegetation 1988. Toxicol Environ Chem 29:73
123. Holst LL, Giesy JP (1989) Chronic effects of the photo enhanced toxicity of anthracene on *Daphnia magna* reproduction. Environ Toxicol Chem 8:933–942
124. Hooftman RN (1991) The toxicity and uptake of chrysene in a reproduction test with *Daphnia magna*. TNO-report IMW-R 91/217
125. Hooftman RN, Evers de Ruiter A (1992) The toxicity and uptake of fluorantnene in *Brachydanio rerio* in an early life stage test. TNO-Rep IMW-R 92/207
126. Hooftman RN, Evers de Ruiter A (1992) The toxicity and uptake of benzo-[*k*]fluoranthene using *Brachydanio rerio* in an early life stage test. TNO-Rep IMW-R 92/218
127. Hooftman RN, Evers de Ruiter A (1992) Early life stage tests with *Brachydanio rerio* and several polycyclic aromatic hydrocarbons using an intermittent flow-through system. TNO-Rep IMW-R 92/210
128. Hooftman RN, Henzen L, Roza P (1993) The toxicity of a polycyclic hydrocarbon mixture in an early life stage toxicity test carried out in an intermittent flow-through system. TNO-Rep IMW-R 9/253
129. Hopkin SP (1993) In situ biological monitoring of pollution in terrestrial and aquatic ecosystems. In: Calow P (ed) Handbook of ecotoxicology-vol I. Blackwell, London
130. Hose JE, Hannah JB, DiJulio D, Landolt ML, Miller BS, Iwaoka WT, Felton SP (1982) Effects of benzo[*a*]pyrene on early development of flatfish. Arch Environ Contam Toxicol 11:167–171
131. Hose JE, Puffer HW, Oshida PS, Bay SM (1983) Developmental and cytogenetic abnormalities induced in the purple sea urchin by environmental levels of benzo[*a*]pyrene. Arch Environ Contam Toxicol 12:319–325
132. Howard PH (Ed.) (1989) Handbook of environmental fate and exposure data for organic chemicals-vol-I: large production and priority pollutants. Lewis, Chelsea, MI
133. Huang XD, Dixon DG, Greenberg BM (1993) Impacts of UV radiation and photomodification on the toxicity of PAHs to the higher plant *Lemna gibba* (duckweed). Environ Toxicol Chem 12:1067–1077
134. Hulzebos EM, Adema DMM, Dirven-van Breemen EM, Henzen L, Van Dis WA, Herbold HA, Hoekstra JA, Baerselman R, Van Gestel CAM (1993) Phytotoxicity studies with *Lactuca sativa* in soil and nutrient solution Environ Toxicol Chem 12:1079–1094
135. Ianuzzi TJ, Bonnevie NL, Huntley SL, Wenning RJ, Trunchon SP, Tull JD (1995) Comments on the use of equilibrium partitioning to establish sediment quality criteria for nonionic chemicals. Environ Toxicol Chem 14:1257–1259
136. IARC (1987) Monographs on the evaluation of the carcinogenic risk of chemical to humans. Vol. 32, Suppl 7. International Agency for Research on Cancer, Lyon
137. IKSR (1993) Statusbericht Rhein–Chemisch-physikalische und biologische Untersuchungen bis 1991. Vergleich Istzustand 1990 – Zielvorgaben. Internationale Kommision zum Schutze des Rheins, Koblenz, FRG (in German)
138. ISO (1992) Soil quality-effects of soil pollution on Collembola (*Folsomia candida*): method for the determination of effects on reproduction
139. James MO (1989) Biotransformation and disposition of PAHs in aquatic invertebrates. In: Varanasi U (ed) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, Florida, pp 69–91
140. James MO (1989) Cytochrome P450 monooxygenases in crustaceans. Xenobiotica 19: 1063–1076
141. James MO, Altman AH, Chung-Li JL, Schell JD (1995) Biotransformation, hepatopancreas DNA binding and pharmacokinetics of benz[*a*]pyrene after oral and parenteral administration to the American lobster, *Homarus americanus*. Chem-Biol Interact 95: 141–160
142. Johnson LL, Casillas E, Collier TK, McCain BB, Varanasi U (1988) Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Can J Fish Aquat Sci 45:2133–2146

143. Johnson LL, Stehr CM, Olson OP, Myers MS, Pierce SM, Wigren C, McCain BB, Varanasi U (1993) Chemical contaminants and hepatic lesions in winter flounder (*Pleuronectes americanus*) from the northeast coast of the United States. Environ Sci Technol 27: 2759–2771
144. Jordan VC (1994) Biochemical pharmacology of antiestrogen action. Pharmacol Rev 38: 245–276
145. Kagan J, Kagan ED (1986) The toxicity of benzo[*a*]pyrene and pyrene in the mosquito *Aedes aegypti*, in the dark and in the presence of ultraviolet light. Chemosphere 15: 243–251
146. Kagan J, Kagan ED, Kagan IA, Kagan PA, Quigley S (1985) The phototoxicity of non-carcinogenic polycyclic aromatic hydrocarbons in aquatic organisms. Chemosphere 14: 1829–1834
147. Kagan J, Kagan PA, Buhse HE Jr (1984) Light-dependent toxicity of a-terthienyl and anthracene toward late embryonic stages of *Rana pipiens*. J Chem Ecology 10 (7):1115–1122
148. Kalf DF, Crommentuijn GH, Posthumus R, Van de Plassche E (1995) Integrated environmental quality objectives for polycyclic aromatic hydrocarbons (PAHs). National Institute of Public Health and the Environment (RIVM), Netherlands, Rep 679 101 018
149. Kalf DF, Crommentuijn T, Van de Plassche E (1997) Environmental quality objectives for 10 polycyclic aromatic hydrocarbons (PAHs). Ecotoxicol Environ Saf 36:89–97
150. Karcher W (1989) Spectral atlas of polycyclic aromatic compounds-vol. 2 – including data on physico-chemical properties and biological activity. Kluwer Academic Publishers, Dordrecht, Netherlands
151. Karickhoff SW, Brown DS, Scott TA (1979) Sorption of hydrophobic pollutants on natural sediments. Water Res 13:241–248
152. Kauss PB, Hutchinson TC (1975) The effects of water-soluble petroleum components on the growth of *Chlorella vulgaris* Beijerinck. Environ Pollut 9:157–174
153. Kayal SI, Connell DW (1990) Partitioning of unsubstituted polycyclic aromatic hydrocarbons between surface sediments and the water column in the Brisbane River estuary. Aust J Freshwat Res 41:443–456
154. Kezíc N, Lucic D, Sulimanovic D (1992) Induction of mixed function hydroxylase in honeybee as a bioassay for detection of environmental xenobiotics. Apidology 23: 217–223
155. Kirchmann H, Astrom H, Jonsall G (1991) Organic pollutants in sewage sludge. Swedisch J Agric Res 21:107–113
156. Könemann H (1981) Quantitative structure-activity relationships in fish toxicity studies. Part I: Relationships for 50 industrial pollutants.Toxicol 19:209–221
157. Könemann H, Van Leeuwen K (1980) Toxicokinetics in fish: accumulation and elimination of six chlorobenzenes by guppies. Chemosphere 9:3–19
158. Korn S, Moles DA, Rice D (1979) Effects of temperature on the median tolerance limit of pink Salmon and schrimp exposed to toluene, naphthalene, and cook inlet crude oil. Bull Environ Contam Toxicol 21:521–525
159. Krahn MM, Burrows DG, MacLeod WD, Malins DC (1987) Determination of individual metabolites of aromatic compounds in hydrolyzed bile of English sole (*Parophrys vetulus*) from polluted sites in Puget Sound, Washington. Arch Environ Contam Toxicol 16: 511–522
160. Krahn MM, Myers MS, Burrows DG, Malins DC (1984) Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. Xenobiotica 14:633–646
161. Krahn MM, Rhodes LD, Myers MS, Moore LK, MacLeod WD, Malins DC (1986) Association between metabolites of aromatic compounds in bile and the occurence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Arch Environ Contam Toxicol 15: 61–67
162. Krieger RI, Feeny PP, Wilkinson CF (1971) Detoxication enzymes in the guts of caterpillars: an evolutionary answer to plant defenses. Science 172:579–580
163. Kulkarni BG, Maserukar VB (1984) Behavioural responses of the crab *Scylla serrata* (Forskal) exposed to naphthalene. J Curr Biosci 1:131

164. Lake J, Hoffman GL, Schimmel SC (1985) Bioaccumulation of contaminants from Black Rock Harbor dredged material by mussels and polychaetes. Tech Rep. D-85-2, U.S. Environmental Protection Agency, Wahington, DC
165. Landahl JT, McCain BB, Myers MS, Rhodes LD, Brown DW (1990) Consistent associations between hepatic lesions in English sole (*Parophrys vetulus*) and polycyclic aromatic hydrocarbons in bottom sediment. Environ Health Perspect 89:195-203
166. Landrum PF (1988) Toxicokinetics of organic xenobiotics in the amphipod, *Pontaporeia hoyi*: role of physiological and environmental variables. Aquat Toxicol 12:245-271
167. Landrum PF, Dupuis WS, Kukkonen J (1994) Toxicokinetics and toxicity of sediment-associated pyrene and phenanthrene in *Diporeia* spp.: examination of equilibirum-partitioning theory and residue based effects for assessing hazards. Environ Toxicol Chem 13:1769-1780
168. Landrum PF, Eadie BJ, Faust WR (1991) Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons. Environ Toxicol Chem 10:35-46
169. Landrum PF, Giesy JP, Oris JT, Allred PMA (1987) Photo induced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms. In: Hrudey JH, Van der Meulen SE (eds) Oil in freshwater: biology, countermeasure technology. Pergamon, New York, pp 304-318
170. Landrum PF, Lee H, Lydy MJ (1992) Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ Toxicol Chem 11:1709-1725
171. Landrum PF, Nihart SR, Eadie BJ, Herche LR (1987) Reduction in bioavailability of organic contaminants to the amphipod *Pontoporeia hoyi* by organic matter of sediment interstitial waters. Environ Toxicol Chem 6:11-20
172. Landrum PF, Poore R (1988) Toxicokinetics of selected xenobiotics in *Hexagenia limbata*. J Gr Lakes Res 14:427-437
173. Landrum PF, Rheinhold MD, Nihart SR, Eadie BJ (1985) Predicting the bioavailability of organic xenobiotics to *Pontaporeia hoyi* in the presence of humic and fulvic materials and natural dissolved organic matter. Environ Toxicol Chem 4: 459-467
174. Landrum PF, Scavia D (1983) Influence of sediment on anthracene uptake, depuration and biotransformation by the amphipod *Hyalella azteca*. Can J Fish Aquat Sci 40: 298-305
175. Lauenstein GG (1995) Comparison of organic contaminants found in mussels and oysters from a current mussel watch project with those from archived mollusc samples of the 1970s. Mar Pollut Bull 30:826-833
176. Laughlin RB Jr, Neff JM (1979) Interactive effects of salinity, temperature and polycyclic aromatic hydrocarbons on the survival and development rate of larvae of the mud crab *Rhithropanopeus harrisii*. Marine Biology 53:281-291
177. Laughlin RB, Neff JM (1981) Ontogeny of respiratory and growth responses of larval mud crabs *Rhithropanopeus harrisii* exposed to different temperatures, salinities and naphthalene concentrations. Marine Ecology 5:319-332
178. Leblanc GA (1980) Acute toxicity of priority pollutants to water flea (*Daphnia magna*). Bull Environ Contam Toxicol 24:684-691
179. Lee H II (1992) Models, muddles, and mud: predicting bioaccumulation of sediment-associated pollutants. In: Burton, GA Jr (ed) Sediment toxicity assessment. Lewis Publishers, Chelsea, MI, pp 267-293
180. Lee H II, Boese BL, Pelletier J, Winsor M, Specht DT, Randall RC (1993) Guidance manual: bedded sediment bioaccumulation tests. EPA 600/R-9_/183, US Environmental Protection Agency, ERL-Narragansett, RI
181. Lee RF (1977) Accumulation and turnover of petroleum hydrocarbons in marine organisms In: Wolfe DA (ed) Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. Pergamon, Oxford New York, pp 60-70
182. Lee RF (1988) Possible linkages between mixed function oxygenase systems, steroid metabolism, reproduction, molting and pollution in aquatic animals. In: Evans MS (ed) Toxic contaminants and ecosystem health: a great lakes focus. Adv Environ Sci Technol 21. Wiley, New York, pp 201-213
183. Lee RF, Ryan C, Neuhauser ML (1976) Fate of petroleum hydrocarbons taken up from food and water by the blue crab *Callinectes sapidus*. Mar Biol 37:363-370

184. Lee WY, Nicol JAC (1978) The effect on naphthalene on survival and activity of the Amphipod *Parhyale*. Bull Environ Contam Toxicol 20:233–240
185. Lemaire P, Den Besten PJ, O'Hara SCM, Livingstone DR (1993) Comparative metobolism of benzo[*a*]pyrene by microsomes of hepatopancreas of the shore crab *Carcinus maenas* L. and digestive gland of the common mussel *Mytilus edulis* L. Polycyclic Aromatic Compounds 3 (suppl): 1133–1140
186. Lemaire P, Lemaire-Gony S, Berhaut J, Lafaurie M (1992) The uptake, metabolism and biological halflife of benzo[*a*]pyrene administered by forcefeeding in sea bass. Ecotox Environ Safety 23:244–251
187. Leversee GJ, Giesy JP, Landrum PF, Gerould S, Bowling JW, Fannin TE, Haddock JD, Bartell SM (1982) Kinetics and biotransformation of benzo[*a*]pyrene in *Chironomus riparius*. Arch Environ Contam Toxicol 11:25–31
188. Levitan WM, Taylor MH (1979) Physiology of salinity-dependent naphthalene toxicity in *Fundulus heteroclitus*. J Fish Res Board Can 36:615–620
189. Lin ELC, Cormier SM, Racine RN (1994) Synchronous fluorometric measurement of metabolites of polycyclic aromatic hydrocarbons in the bile of brown bullhead. Environ Toxicol Chem 13:707–715
190. Lipnick RL (1989) A quantitative structure-activity relationship study of Overton's data on the narcosis and toxicity of organic compounds to the tadpole, *Rana temporia*. In: Suter II GW, Lewis MA (eds) Aquatic toxicology and environmental fate vol 11. ASTM, Philidelphia, pp 468–489
191. Livingstone DR (1993) Biotechnology and pollution monitoring: use of molecular biomarkers in the aquatic environment. J Chem Tech Biotechnol 57:195–211
192. Livingstone DR, Farrar SV (1984) Tissue and subcellular distribution of enzyme activities of mixed-function oxygenase and benzo[*a*]pyrene metabolism in the common mussel *Mytilus edulis* L. Sci Tot Environ 39:209–235
193. Livingstone DR, Kirchin MA, Wiseman A (1989) Cytochrome P-450 and oxidative metabolism in molluscs. Xenobiotica 19:1041–1062
194. Logan DT, Wilson HT (1995) An ecological risk assessment method for species exposed to contaminant mixtures. Environ Toxicol Chem 14:351–359
195. Long ER, MacDonald DD, Smith SL, Calder FD (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Env Managem 19:81–97
196. Loonen H (1994) Bioavailability of chlorinated dioxins and furans in the aquatic environment. PhD Thesis, University of Amsterdam, Netherlands
197. Lu P-Y, Metcalf RL, Plummer N, Mandel D (1977) The environmental fate of three carcinogens: benzo[*a*]pyrene, benzidine, and vinyl chloride evaluated in laboratory model ecosystems. Arch Environ Contam Toxicol 6:129–142
198. Mackay D (1982) Correlation of bioconcentration factors. Environ Sci Technol 16: 274–278
199. Malins DC, McCain BB, Myers MS, Brown DW, Krahn MM, Chan SL, Roubal WT (1988) Neoplastic and other diseases in fish in relation to toxic chemicals: an overview. Aquat Toxicol 11:43–67
200. Markwell RD, Connell DW, Gabric AJ (1989) Bioaccumulation of lipophilic compounds from sediments by oligochaetes. Water Res 23:1443–1450
201. Marsh JW, Chipman JK, Livingstone DR (1992) Activation of xenobiotics to reactive and mutagenic products by the marine invertebrates *Mytilus edulis*, *Carcinus maenas* and *Asterias rubens*. Aquat Toxicol 22:115–128
202. McCarthy JF, Jimenez BD (1985) Reduction in bioavailability to bluegills of polycyclic aromatic hydrocarbons. Environ Toxicol Chem 4:511–521
203. McCarthy JF, Jimenez BD, Barbee T (1985) Effect of dissolved humic material on accumulation of polycyclic aromatic hydrocarbons: structure-activity relationships. Aquat Toxicol 7:15–24
204. McCarty LS (1991) Toxicant body residues: implications for aquatic bioassays with some organic chemicals. In: Mayes MA, Barron MG (eds) Aquatic toxicology and risk assessment (14). Am Soc Test Mat, Philidelphia, pp 183–192

205. McCarty LS, Mackay D (1993) Enhancing ecotoxicological modeling and assessment. Environ Sci Technol 11:917–930
206. McCarty LS, Mackay D, Smith AD, Ozburn GW, Dixon DG (1992) Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: neutral narcotic organs. Environ Toxicol Chem 11:917–930
207. McCloskey JT, Oris JT (1991) Effect of water temperature and dissolved oxygen concentration on the photo-induced toxicity of anthracene to juvenile bluegill sunfish (*Lepomis macrochirus*). Aquat Toxicol 21:145–156
208. McElroy AE (1990) Polycyclic aromatic hydrocarbon metabolism in the polychaete *Nereis virens*. Aquat Toxicol 18:35–50
209. McElroy AE, Farrington JW, Teal JM (1989) Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment. In: Varanasi U (ed) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, Florida, pp 1–40
210. McLeese DW, Burridge LE (1987) Comparative accumulation of PAHs in four marine invertebrates. In: Capuzzo JM, Kester DR (eds) Oceanic processes in marine pollution-volume 1. RE Krieger, Malabar, FA, pp 109–117
211. Meador JP, Casillas E, Sloan CA, Varanasi U (1995) Comparative bioaccumulation of polycyclic aromatic hydrocarbons from sediment by two infaunal invertebrates. Mar Ecol Prog Ser 123: 107–124
212. Meador JP, Stein JE, Reichert WL, Varanasi U (1995) Bioaccumulation of polycyclic aromatic hydrocarbans by marine organisms. Rev Environ Contam Toxicol 143:79–165
213. Means JC, Wood SG, Hassett JJ, Banwart WL (1980) Sorption of polynuclear aromatic hydrocarbons by sediments and soils. Environ Sci Technol 14:1524–1528
214. MEDCHEM (1992) Database and calculation method for log Kow values. Developed at Pomona College, Claremont, CA
215. Mekenyan OG, Ankley GT, Veith GD, Call DJ (1994) QSARs for photoinduced toxicity. 1. Acute lethality of polycyclic aromatic hydrocarbons to *Daphnia magna*. Chemosphere 28:567–582
216. Millem... RE, Birge WJ, Black JA, Cushman RM, Daniels KL, Franco PJ, Giddings JM, McCarthy JF, Stewart AJ (1984) Comparative acute toxicity to aquatic organisms of components of coal derived synthetic fuels. Trans Am Fish Soc 113:74–85
217. Mitchell RL, Burchett MD, Pulkownik A, McCluskey (1988) Effects of environmentally hazardous chemicals on the emergence and early growth of selected Australian plants. Plant Soil 112:195–199
218. Mix MC (1986) Cancerous diseases in aquatic animals and their association with environmental pollutatants: a critical literature review. Mar Environ Res 20:1–141
219. Moles A (1980) Sensitivity of parasitized coho salmon fry to crude oil, toluene and naphthalene. Trans Am Fish Soc 109:293–297
220. Moles A, Bates S, Rice SD, Korn S (1981) Reduced growth of coho salmon fry exposed to two petroleum components toluene and naphthalene in fresh water. Trans Am Fish Soc 110:430–436
221. Moles A, Rice SD (1983) Effects of crude oil and naphthalene on growth, caloric content and fat content of pink salmon juveniles in seawater. Trans Am ... sh Soc 112:205
222. Monosson E, Stegeman. JJ (1991) Cytochrome P450 E (P4501 A) induction and inhibition in winter flounder by 3,3′,4,4′-tetrachlorobiphenyl: comparison of response in fish from Georges Bank and Narragansett Bay. Environ Toxicol Chem 10:765–774
223. Monson PD, Ankley GT, Kosian PA (1995) Phototoxic response of *Lumbriculus variegatus* to sediments contaminated by polycyclic aromatic hydrocarbons–short communication. Environ Toxicol Chem 14:891–894
224. Müller AMF, Makropoulos V, Bolt HM (1995) Toxicological aspects of oestrogen-mimetic xenobiotics present in the environment. Toxicol Ecotoxicol News 2:68–73
225. Mullin CA (1985) Detoxification enzyme relationships in arthropods of differing feeding strategies. In: Hedin PA (ed) Bioregulators for pest control. ACS Symposium series 276: 267–278

226. Munoz, MJ, Tarazona JV (1993) Synergistic effect of two- and four-component combinations of the polycyclic aromatic hydrocarbons: phenanthrene, anthracene, naphthalene and acenaphthene on *Daphnia magna*. Bull Environ Contam Toxicol 50:363–368
227. Myers MS, Stehr CM, Olson OP, Johnson LL, McCain BB, Chan S-L, Varanasi U (1994) Relationships between toxopathic hepatic lesions and exposure to chemical contaminants in English sole (*Pleuronectes vetulus*), starry flounder (*Platichthys stellatus*), and white croaker (*Genyonemus lineatus*) from selected marine sites on the pacific coast, USA. Environ Health Perspect 102:2–17
228. Narbonne JF, Garrigues P, Ribera D, Raoux C, Mathieu A, Lemaire P, Salaun JP, Lafaurie M (1991) Mixed-function oxygenase enzymes as tools for pollution monitoring: field studies on the French coast of the Mediterranean Sea. Comp Biochem Physiol 100 C: 37–42
229. Neely WB, Branson DR, Blau GE (1974) Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ Sci Technol 8:1113–1115
230. Neff JM (1979) Polycyclic aromatic hydrocarbons in the aquatic environment. Applied Science Publishers, London
231. Neff JM (1984) Bioaccumulation of organic micropollutants from sediments and suspended particulates by aquatic animals. Fresenius Z Anal Chem 319:132–136
232. Newsted JL, Giesy JP (1987) Predictive models for photoinduced toxicity of polycyclic aromatic hydrocarbons to *Daphnia magna*, Strauss (Cladocera, Crustacea). Environ Toxicol Chem 6:445–461
233. Niaussat P, Auger C (1970) Mise en évidence et répartition du benzo-3–4-pyrène et du pérylène chez différents organismes de la biocoenose lagunaire de Clipperton. CR Acad Sci Paris 270(d):2702–2705
234. Niimi AJ, Dookhran GP (1989) Dietary absorption efficiencies and elimination rates of polycyclic aromatic hydrocarbons (PAHs) in rainbow trout (*Salmo gairdneri*). Environ Toxicol Chem 8:719–722
235. Niimi AJ, Palazzo V (1986) Biological half-lives of eight polycyclic aromatic hydrocarbons (PAHs) in rainbow trout (*Salmo gairdneri*). Water Res 20:503–507
236. Nisbet ICT, LaGoy PK (1992) Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regulat Toxicol Pharmacol 16:290–300
237. NSTF–North Sea Task Force (1994) North Sea subregion 4. Assessment report 1993. Oslo and Paris Commissions, International Council for the Exploration of the Sea
238. O'Connor TP, Cantillo AY, Lauenstein GG (1994) Monitoring of temporal trends in chemical contamination by the NOAA national status and trends mussel watch project. In: Kramer KJM (ed) Biomonitoring of coastal waters and estuaries. CRC Press, Boca Raton, Ann Arbor, London, Tokyo, pp 29–50
239. OECD (1984) Guidelines for testing of chemicals, Section 2, effects on biotic systems: alga growth inhibition test. no. 201, Adopted 7 June, 1984
240. OECD (1984) Guidelines for testing of chemicals, Section 2, effects on biotic systems: earthworm, acute toxicity tests. no. 207, Adopted 4 April, 1984
241. OECD (1992) Guidelines for testing of chemicals, Section 2, effects on biotic systems: fish, early-life stage toxicity test. no. 210, Adopted 17 July, 1992
242. OECD (1992) Report of the OECD workshop on effects of chemicals in sediment (Copenhagen 13–15 May 1991) Environment monographs no 60. Paris 1992
243. Oliver BG (1985) Desorption of chlorinated hydrocarbons from spiked and anthropogenically contaminated sediments. Chemosphere 14:1087–1106
244. Oliver BG (1987) Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. Environ Sci Technol 21:785–790
245. Oliver BG, AJ Niimi (1988) Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environ Sci Technol 22:388–397
246. Opperhuizen A, Sijm DTHM (1990) Bioaccumulation and biotransformation of polychlorinated dibenzo-p-dioxins and dibenzofurans in fish. Environ Toxicol Chem 9: 175–186

247. Opperhuizen A, Velde EW, Gobas FAPC, Liem DAK, Steen JMD (1985) Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. Chemosphere 14:1871
248. Oris JT, Giesy JP (1985) The photoenhanced toxicity of anthracene to juvenile sunfish (*Lepomis* spp.). Aquat Toxicol 6:133–146
249. Oris JT, Giesy JP (1987) The photoinduced toxicity of polycyclic aromatic hydrocarbons to larvae ot the fathead minnow (*Pimephales promelas*). Chemosphere 16:1395–1404
250. Oris JT, Hall AT, Tylka JD (1990) Humic acids reduce the photo induced toxicity of anthracene to fish and *Daphnia*. Environ Toxicol Chem 9:575–583
251. Oris JT, Giesy JP, Allred PM (1984) Photoinduced toxicity of anthracene in aquatic organisms, an environmental perspective. In: Veziroglu (ed) The biosphere: problems and solutions. Elsevier Science Publ. B. V., Amsterdam, Netherlands
252. Ott FS, Harris RP, O'Hara SCM (1978) Acute and sublethal toxicity of naphthalene and three methylated derivates to the estuarine copepod, *Eurytemora affinis*. Marine Env Res 1:49–58
253. Paine MD, Chapman PM, Allard PJ, Murdoch MH, Minifie D (1996) Limited bioavailability of sediment PAH near an aluminium smelter: contamination does not equal effects. Environ Toxicol Chem 15:2003–2018
254. Parkerton TF (1993) Estimating toxicokinetic parameters for modeling the bioaccumulation of non-ionic organic chemicals in aquatic organisms. PhD-Thesis, Graduate school–New Brunswick Rutgers, State University of New Jersey
255. Passino DRM, Smith SB (1987) Acute bioassays and hazard evaluation of representative contaminants detected in Great Lakes fish. Environ Toxicol Chem 6:901–907
256. Payne JF, Fancey LL, Rahimtula AD, Porter EL (1987) Review and perspective on the use of mixed function oxygenase enzymes in biological monitoring. Comp Biochem Physiol 86 C:233–245
257. Payne JF, Kiceniuk J, Fancey LL, Williams U, Fletcher GL, Rahimtula A, Fowler B (1988) What is a safe level of polycyclic aromatic hydrocarbons for fish: subchronic toxicity study on winter flounder (*Pseudopleuronectes americanus*)? Can J Fish Aquat Sci 45: 1983–1993
258. Peachy RL, Crosby DG (1996) Phototoxicity in tropical reef animals. Mar Environ Res 42: 359–362
259. Peakall D (1994) The role of biomarkers in environmental assessment. 1 Introduction. Ecotoxicology 3:157–160
260. Peakall DB, Shugart LR (1990) Biomarkers: research and application in the assessment of environmental health. Springer, Berlin, Heidelberg New York
261. Pongs O (1989) Biochemical properties of ecdysteroid receptors. In: Koolman J (ed) Ecdysone, from chemistry to mode of action. Thieme, Stutgart New York
262. Pruell RJ, Quinn JG, Lake JL, Davis WR (1987) Availability of PCBs and PAHs to *Mytilus edulis* from artificially resuspended sediments. In: Capuzzo JM, Kester DR (eds) Oceanic processes in marine pollution, vol. 1. Biological processes and wastes in the ocean. RE Krieger, Malabar, FL, pp 97–108
263. Pruell RJ, Rubinstein NI, Taplin BK, Livolsi JA, Bowen RD (1993) Accumulation of polychlorinated organic contaminants from sediment by 3 benthic marinespecies. Arch Environ Contam Toxicol 24:290–297
264. QSR (1993) North sea quality status report 1993. North Sea Task Force, OSPARCOM, ICES, London.
265. Readman JW, Mantoura RFC, Rhead MM (1984) The physico-chemical speciation of polycyclic aromatic hydrocarbons (PAH) in aquatic systems. Fres Z Anal Chem 319, 126–131
266. Reichert WL, Le Eberhart BT, Varanasi U (1985) Exposure of two species of deposit-feeding amphipods to sediment-associated [3H]benzo[*a*]pyrene: uptake, metabolism and covalent binding to tissue macromolecules. Aquat Toxicol 6:45–56
267. Remde A, Hund K (1994) Response of soil autotrophic nitrification and soil respiration to chemical pollution in long-term experiments. Chemosphere 29:391–404

268. Ren L, Huang XD, McConkey BJ, Dixon DG, Greenberg BM (1994) Photoinduced toxicity of three polycyclic aromatic hydrocarbons (fluoranthene, pyrene and naphthalene) to the duckweed *Lemna gibba* L. Ecotox Environ Saf 28:160–171
269. Rogerson A, Ying Shiu A, Lan Huang G, Mackay D, Berger J (1983) Determination and interpretation of hydrocarbon toxicity to ciliate protozoa. Aquat Toxicol 3:215–228
270. Ronis MJJ, Hansson T, Borlakoglu J, Walker CH (1989) Cytochromes P-450 of seabirds: cross reactivity studies with purified rat cytochromes. Xenobiotica 19:1167–1173
271. Ronis MJJ, Hodgson E (1989) Cytochrome P-450 monooxygenases in insects. Xenobiotica 19:1077–1092
272. Rossi SS, Neff JM (1978) Toxicity of polynuclear aromatic hydrocarbons to the polychaete *Neanthes arenaceodentata*. Mar Poll Bull 9:220–223
273. Sabourin TD (1982) Respiratory and circulatory responses of the blue crab to naphthalene and the effect of acclimation salinity. Aquat Toxicol 2:301–318
274. Saethre LJ, Falf-Petersen IB, Sydnes LK, Lonning S, Naley AM (1984) Toxicity and chemical reactivity of naphthalene and methylnaphthalenes. Aquat Toxicol 5:291–306
275. Sanborn HR, Malins DC (1977) Toxicity and metabolism of naphthalene: A study with marine larval invertebrates Proc Soc Exp Biol Med 154:151–155
276. Savino JF, Tanabe LL (1989) Sublethal effects of phenanthrene, nicotine, and pinane on *Daphnia pulex*. Bull Environ Contam Toxicol 42:778–784
277. Schoeny R, Cody T, Warshawsky D, Radike M (1988) Metabolism of mutagenic polycyclic aromatic hydrocarbons by photosynthtic algae species. Mutat Res 197:289–302
278. Schrap SM, Opperhuizen A (1989) Quantifying the sorption of organic chemicals on sediments. Chemosphere 18:1883–1893
279. Schrap SM, Opperhuizen A (1990) Relationship between bioavailability and hydrophobicity: reduction of the uptake of organic chemicals by fish due to the sorption on particles. Environ Toxicol Chem 9:715–724
280. Serano JL, Wade TL, Brooks JM, Atlas EL, Fay RR, Wilkinson DL (1993) National status and trends of mussel watch program, chlordane-related compounds in Gulf of Mexico oysters, 1986–1990. Environ Pollut 82:23–32
281. Shea D (1988) Developing national sediment quality criteria–equilibrium partitioning of contaminants as a means of evaluating sediment quality Environ Sci Technol 22: 1256–1261
282. Shorten CV, Elzerman AW, Mills GL (1990) Methods for the determination of PAH desorption kinetics in coal. Chemosphere 20:137–159
283. Siegfried BD, Young LJ (1993) Activity of detoxification enzymes in aquatic and terrestrial insects. Environ Entomol 22:958–963
284. Sijm DTHM, Opperhuizen A (1988) Biotransformation, bioaccumulation and lethality of 2,8-dichlorodibenzo-*p*-dioxin, a proposal to explain the biotic fate and toxicity of PCDDs and PCDFs. Chemosphere 17:83–99
285. Sikka HC, Rutkowski JP, Kandaswami C (1990) Comparative metabolism of benz[*a*]pyrene by liver microsomes from brown bullhead and carp. Aquat Toxicol 16:101–112
286. Sikka HC, Steward AR, Kandaswami C, Rutkowski JP, Zaleski J, Kumar S, Earley K, Gupta RC (1991) Metabolism of benzo[*a*]pyrene and persistence of DNA adducts in the brown bullhead (*Ictalurus nebulosus*). Comp Biochem Physiol 100 C:25–28
287. Sims RC, Overcash MR (1983) Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. Residue Rev 88:1–68
288. Sleiderink HM, Beyer J, Scholtens E, Goksøyr A, Nieuwenhuize J, Van Liere JM, Everaarts JM, Boon JP (1995) Influence of temperature and polyaromatic contaminants on CYP1 A levels in North Sea dab (*Limanda limanda*). Aquat Toxicol 32:189–209
289. Slooff J, Janus JA, Matthijsen AJCM, Montizaan GK, Ros JPM (1989) Integrated criteria document PAHs. National Institute of Public Health and the Environment (RIVM), Netherlands, Rep 758474 011
290. Smith RL, Hargreaves BR (1983) A simple toxicity apperatus for contineous flow with small volumes: demonstration with mysids and naphthalene. Bull Environ Contam Toxicol 30:406–412

291. Smith SB, Savino JF, Blouin MA (1988) Acute toxicity to *Daphnia pulex* of six classes of chemical compounds potentially hazardous to great lakes aquatic biota. J Great Lakes Res 14:394–404
292. Southworth GR, Beauchamp JJ, Schmieder PK (1978) Biaccumulation potential of polycyclic aromatic hydrocarbons in *Daphnia pulex*. Water Res 12:973–977
293. Spacie A, Hamelink JL (1983) Alternative models for describing the bioconcentration of organics in fish. Environ Toxicol Chem 1:309–320
294. Spacie A, Hamelink JL (1985) Bioaccumulation In: Rand GM, Petrocelli SR (eds) Fundamentals of aquatic toxicology. Hemisphere, Washington, pp. 495–525
295. Stagg RM, McIntosh A (in press) Determination of CYP1 A dependent mono-oxygenase activity in the liver of the dab (*Limanda limanda* L) by the fluorimetric measurement of 7-ethoxyresorufin-*O*-deethylase (EROD) activity. ICES TIMES
296. Stegeman JJ (1985) Benzo[*a*]pyrene oxidation and microsomal enzyme activity in the mussel (*Mytilus edulis*) and other bivalve mollusc species from the Western North Atlantic. Mar Biol 89:21–30
297. Stegeman JJ (1989) Cytochrome P450 forms in fish: catalytic, immunological and sequence similarities. Xenobiotica 19:1093–1110
298. Stegeman JJ, Lech JJ (1991) Cytochrome P-450 Monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ Health Persp 90:101–109
299. Stegeman JJ, Woodin BR, Goksøyr A (1988) Apparent cytochrome P450 induction as an indication of exposure to environmental chemicals in the flounder *Platichthys flesus*. Mar Ecol Prog Ser 46:55–60
300. Stehly GR, Landrum PF, Henry MGM, Klemm C (1990) Toxicokinetics of PAH in *Hexagenia*. Environ Toxicol Chem 9:167–174
301. Stein JE, Reichert WL, French B, Varanasi U (1993) 32P-Postlabelling analysis of DNA adduct formation and persistence in English sole (*Pleuronectes vetulus*) exposed to benzo[*a*]pyrene and 7H-dibenzo[*c,g*]carbazole. Chem Biol Interact 88:55–69
302. Stenersen J, Kobro S, Bjerke MUA (1987) Glutathione transferases in aquatic and terrestrial animals from nine phyla. Comp Biochem Physiol 86 C:73–82
303. Stronkhorst J (1992) Trends in pollutants in blue mussel *Mytilus edulis* and flounder *Platichthys flesus* from two Dutch estuaries, 1985–1990. Mar Pollut Bull 24:250–258
304. Stroomberg GJ, Reuther C, Kozin I, Van Brummelen TC, Van Gestel CAM, Gooijer C, Cofino WP (1996) Formation of pyrene metabolites by the terrestrial isopod *Porcellio scaber*. Chemosphere 33:1905–1914
305. Suedel BC, Rodgers JH, Clifford PA (1993) Bioavailability of fluoranthene in freshwater sediment toxicity tests. Environ Toxicol Chem 12:155–156
306. Sugatt RH, O'Grady DP, Banerjee S (1984) Toxicity of organic mixtures saturated with water to *Daphnia magna*. Effect of compositional changes. Chemosphere 13:11–12
307. Swartz RC, Schults DW, Ozretich RJ, Lamberson JO, Cole FA, DeWitt TH, Redmond MS, Ferraro SP (1995) PAH: a model to predict the toxicity of polynuclear aromatic hydrocarbon mixtures in field-collected sediments. Environ Toxicol Chem 14:1977–1987
308. Thomann RV, Connolly JP, Parkerton TF (1992) An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. Environ Toxicol Chem 11:615–630
309. Thomas RE, Rice SD (1979) The effect of exposure temperatures on oxygen consumption and opercular breathing rates of pink salmon fry exposed to toluene, naphthalene and water soluble fractions of cook inlet crude oil no. 2 fuel oil. In: Verberg Marine pollution: functional processes. Academic Press, New York
310. Thursby GB, Steele RL, Kane ME (1985) Effect of organic chemicals on growth and reproduction in the marine red algae *Champia parvula*. Environ Toxicol Chem 4:797–805
311. Timbrell JA (1991) Principles of biochemical toxicology. Taylor and Francis, London Washington DC
312. Trucco RG, Engelhardt FR, Stacey B (1983) Toxicity, accumulation and clearance of aromatic hydrocarbons in *Daphnia pulex*. Environ Pollut A 31:191–202

313. Truscott B, Walsh JM, Burton MP, Payne JF, Idler DR (1983) Effect of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. Comp Biochem Physiol 75 C:121–130
314. US Environmental Protection Agency (1993) Sediment quality criteria for the protection of benthic organisms: Fluoranthene. EPA 822-R-93–012. Office of Water, Office of Research and Development, and Office of Science and Technology, Health and Ecological Criteria Division, Washington DC
315. Vaishnav DD, Korthals ET (1990) Comparative toxicities of selected industrial chemicals to microorganisms and other aquatic organisms. Arch Environ Contam Toxicol 19:624–628
316. Van Brummelen TC, Stuijfzand SC (1993) Effects of benzo[*a*]pyrene on survival, growth and energy reserves in the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. Sci Tot Environ S 1993:921–930
317. Van Brummelen TC, Van Gestel CAM, Verweij RA (1996) Long-term toxicity of five polycyclic aromatic hydrocarbons for the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. Environ Toxicol Chem 15:1199–1210
318. Van Brummelen TC, Van Straalen NM (1996) Uptake and elimination of benzo[*a*]pyrene in the terrestrial isopod *Porcellio scaber*. Arch Environ Contam Toxicol 31:277–285
319. Van Brummelen TC, Verweij RA, Wedzinga SA, Van Gestel CAM (1996) Enrichment of polycyclic aromatic hydrocarbons in forest soil near a blast furnace plant. Chemosphere 32:293–314
320. Van Brummelen TC, Verweij RA, Wedzinga SA, Van Gestel CAM (1996) Polycyclic aromatic hydrocarbons in earthworms and isopods from contaminated forest soil. Chemosphere 32:315–341
321. Van Brummelen TC, Verwey RA, Van Straalen NM (1991) Determination of benzo[*a*]pyrene in isopods (*Porcellio scaber* Latr.) exposed to contaminated food. Comp Biochem Physiol 100 C:21–24
322. Van der Kooij LA, Van de Meent D, Van Leeuwen CJ (1991) Deriving quality criteria from water and sediment from the results of aquatic toxicity tests and product standards, application of the equilibrium partitioning method. Water Res 25:697–705
323. Van der Oost R, Heida H, Opperhuizen A (1987) Polychlorinated biphenyl congeners in sediments, plankton, molluscs, crustaceans, and eel in a freshwater lake: implications of using reference chemicals and indicator organisms in bioaccumulation studies. Arch Environ Contam Toxicol 17:721–729
324. Van der Oost R, Van Gastel L, Worst D, Hanraads M, Satumalay K, Van Schooten FJ, Heida H, Vermeulen NPE (1994) Biochemical markers in feral roach (*Rutilus rutilus*) in relation to the bioaccumulation of organic trace pollutants. Chemosphere 29:801–817
325. Van der Oost R, Van Schooten FJ, Ariese F, Heida H, Satumalay K, Vermeulen NPE (1994) Bioaccumulation, biotransformation and DNA binding of PAHs in feral eel (*Anguilla anguilla*) exposed to polluted sediments–a field survey. Environ Toxicol Chem 13:859–870
326. Van Gestel CAM, Ma WC (1988) Toxicity and bioaccumulation of chlorophenols in earthworms in relation to bioavailability in soil. Ecotox Environ Safety 15:289–297
327. Van Gestel CAM, Ma WC (1990) An approach to quantitative structure-activity relationships (QSARs) in earthworm toxicity studies. Chemosphere 21:1023–1033
328. Van Gestel CAM, Van Brummelen TC (1996) Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. Ecotoxicol 5:217–225
329. Van Hattum B (1995) Bioaccumulation of sediment-bound contaminants by the freshwater isopod *Asellus aquaticus*. PhD Thesis. Institute for Environmental Studies, Vrije Universiteit, Amsterdam, Netherlands
330. Van Hattum B, Burgers I, Swart K, Van der Horst A, Wegener JW, Leonards P, Rijkeboer M, Den Besten P (1996) Biomonitoring of micropollutants in food chains in Hollandsch Diep, the Dordtsche and Brabantsche Biesbosch. Institute for Environmental Studies, Vrije Universiteit, Amsterdam, Netherlands Rep E-96/12 (in Dutch)
331. Van Hattum B, Leonards P, Burgers I, Van der Horst B (1993) Micropollutants in organisms from the River Nieuwe Merwede and Dordtse Biesbosch. Institute for Environmental Studies, Vrije Universiteit, Amsterdam, the Netherlands Rep E-92/19 (in Dutch)

332. Van Leeuwen CJ, Hermens JLM (1995) Risk assessment of chemicals. Kluwer Academic Publishers, Dordrecht, ISBN 0-7923-3740-9
333. Van Leeuwen CJ, Van der Zandt PTJ, Aldenberg T, Verhaar HJ, Hermens JL (1992) Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. I Narcotic industrial chemicals. Environ Toxicol Chem 11:267-282
334. Van Straalen NM (1994) Biodiversity of ecotoxicological responses in animals. Netherlands J Zool 44:112-129
335. Van Straalen NM, RA Verweij (1991) Effects of benzo[*a*]pyrene on food assimilation and growth efficiency in *Porcellio scaber* (Isopoda). Bull Environ Contam Toxicol 46:134-140
336. Van Straalen NM, Van Gestel CAM (1993) Soil invertebrates and micro-organisms. In: Calow P (ed) Hanbook of ecotoxicology, vol 1. Blackwell, London, pp 251-277
337. Van Straalen NM, Verweij RA, Van Brummelen TC (1993) PAH concentrations in forest floor invertebrates in the vicinity of a blast furnace plant. In: Garrigues P, Lamotte M (eds) Polycyclic aromatic compounds synthesis, properties, measurements, occurence and biological effects. Polycycl Arom Compounds 3 - supplement. Gordon and Breach Science Publishers, Yverdon, Switzerland , pp 1001-1006
338. Van Welie RTH, Van Dijck RGJM, Vermeulen NPE, Van Sittert NJ (1992) Mercapturic acids, protein adducts, and DNA adducts as biomarkers of electrophilic chemicals. Crit Rev Toxicol 22:271-306
339. Van Wezel AP, Opperhuizen A (1995) Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanisms and membrane burdens. Crit Rev Toxicol 25:225-279
340. Varanasi U, Gmur DJ (1980) Metabolic activation and covalent binding of benzo[*a*]-pyrene to deoxyribonucleic acid catalyzed by liver enzymes of marine fish. Biochem Pharmacol 29:753-761
341. Varanasi U, Nishimoto M, Reichert WL, Stein JE (1982) Metabolism and subsequent covalent binding of benzo[*a*]pyrene to macromolecules in gonads and liver of ripe English sole (*Parophrys vetulus*). Xenobiotica 12:417-425
342. Varanasi U, Reichert WL, Stein JE, Brown DW, Sanborn HR (1985) Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. Environ Sci Technol 19:838-841
343. Varanasi U, Stein JE, Nishimoto M (1989) Biotransformation and disposition of PAH in fish. In: Varanasi U (ed) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, Florida, pp 93-150
344. Veith GD, Call DJ, Brooke LT (1983) Structure-toxicity realationships for the fathead minnow, *Pimephales promelas*: Narcotic industrial chemicals. Can J Fish Aquat Sci 40: 743-748
345. Veith GD, DeFoe DL, Bergstedt BV (1979) Measuring and estimating the bioconcentration factor of chemicals in fish. J Fish Res Board Can 36:1040-1048
346. Verhaar HJM, Leeuwen CJV, Hermens JL (1992) Classifying environmental pollutants. Structure-activity relationships for prediction of aquatic toxicity. Chemosphere 25: 471-491
347. Vethaak AD, ap Rheinallt T (1992) Fish disease as a monitor for marine pollution: the case of the North Sea. Rev Fish Biol Fish 2:1-32
348. Vethaak, AD (1993) Fish disease and marine pollution. Thesis, Universiteit van Amsterdam, Amsterdam
349. Wade TL, Atlas EL, Brooks JM, Kennicutt II MC, Fox RG, Sericano J, Garcia-Romero B, DeFreitas D (1988) NOAA Gulf of Mexico status and trends program: Trace organic contaminant distribution in sediments and oysters. Estuaries 11:171-179
350. Walker CH (1978) Species differences in microsomal monooxygenase activity and their relationship to biological halflives. Drug Metabol Rev 7:295-323
351. Walker CH (1980) Species variations in some hepatic microsomal enzymes that metabolize xenobiotics. Prog Drug Metabol 5:113-164
352. Walker CH, Ronis MJJ (1989) The monooxygenases of birds, reptiles and amphibians. Xenobiotica 19:1111-1121

353. Walton BT (1980) Influence of route of entry on toxicity of polycyclic aromatic hydrocarbons to the cricket (*Acheta demesticus*). Bull Environ Contam Toxicol 25:289–293
354. Waxman DJ (1988) Interactions of hepatic cytochromes P-450 with steroid hormones. Biochem Pharmacol 37:71–84
355. Weissenfels WD, Klewer HJ, Langhoff J (1992) Adsorption of polcyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity. Appl Microbiol Biotechnol 36:689–696
356. Weston DP (1990) Hydrocarbon Bioaccumulation from contaminated sediment by the deposit-feeding polychaete *Abarenicola pacifica*. Mar Biol 107:159–169
357. Wu S, Gschwend PM (1986) Sorption kinetics of hydrophobic organic compounds to natural sediments and soils. Environ Sci Technol 20:717–725